ON THE PRODUCTION OF PLASMA FIBRINOLYTIC ACTIVITY WITHIN VEINS

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INTRODUCTION

"H-m-m-ph! A platelet thrombus! No fibrin! No fibrin!
Ask yourself the question, boy: 'Why is there no fibrin
if platelets are so important in clotting?'"

The late Professor John Shaw Dunn
on demonstrating to the writer,
then an undergraduate, a platelet
thrombus on a valve of a vein.

INTRODUCTION

It has been established clearly that blood platelets rapidly agglutinate at the site of injury to a blood vessel to form an intravascular platelet or 'white' thrombus (3 to 6, 10, 14, 15). Wintrobe (11) refers to this as "their most significant function". Haemostasis by platelet agglutination was first demonstrated by Hayem (7) in an experimental wound of a dog's jugular vein and by Lubnitsky (8) in similar wounds of femoral arteries of rabbits. These observations were later confirmed (1, 13). Zucker (12) and McFadzean (9) independently conducted a histological study of fresh puncture wounds of human skin excised during surgical operations and Apitz (2) reported a similar study of material obtained at necropsy. They found that wounds in veins ranging from post-capillary venules to those some 250 μ in diameter were sealed invariably by platelet thrombi. Injured arterioles were sealed similarly. Fibrin formed outside the injured vessels in the wound track. McFadzean (9) failed to identify injured true capillaries in his preparations. Apitz (2) and Zucker (12) reported that fibrin sealed the mouths of such cut capillaries as were identified and that in no instance did they contain platelet thrombi.

The platelet thrombi in many of the larger venules had intravascular projections which reduced greatly (9, 12) or completely obstructed (9) their lumen. In each instance in which the examination could be carried out the lumen of the vessel on either side of the obstruction was patent and contained no fibrin. A possible explanation for the absence of fibrin in such vessels, especially from the segment proximal in the line of venous flow to the obstructing platelet thrombus, was suggested by a happy coincidence. A patient with cirrhosis of the liver was observed to develop plasma fibrinolytic activity of such intensity as to lyse fibrin as rapidly as it was being formed in the process of coagulation of the blood in vitro. Could fibrin have been formed and lysed in the venules

obstructed by platelet thrombi? Could fibrinolytic activity be stimulated in the plasma of blood within veins? The attempt to answer these questions initiated the observations which are the subject matter of this thesis.

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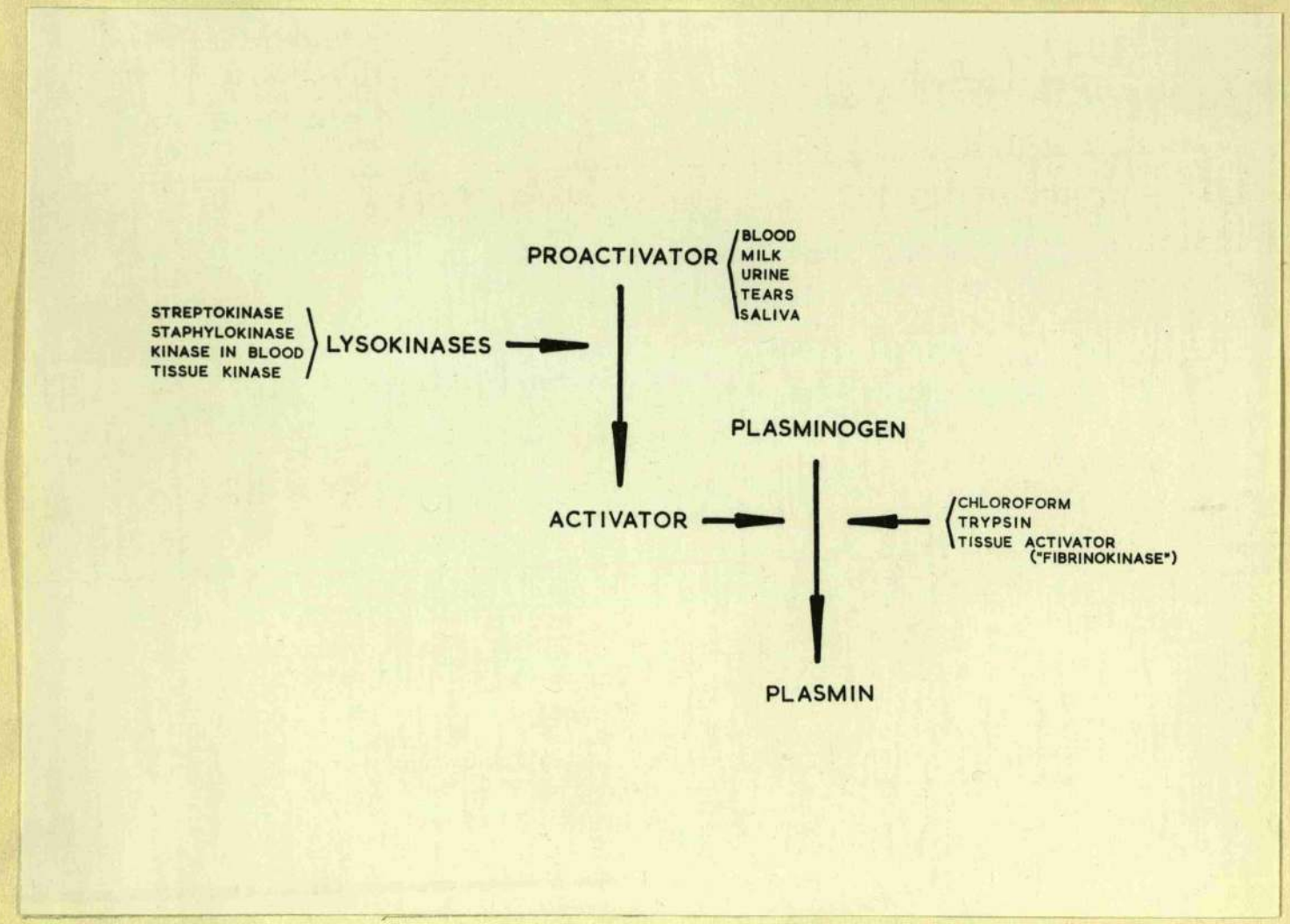

Figure 1. The "activation scheme of 1954", Astrup (4).

Chapter 1

THE FIBRINOLYTIC SYSTEM

Fibrinolysis in Vitro

A proteolytic enzyme capable of fibrinolysis occurs in normal plasma in the form of an inactive precursor, plasminogen, which is associated with the globulin fraction. An inhibitor, antiplasmin is present in the albumin fraction. Plasmin produces not only fibrinolysis but digests other plasma proteins, including fibrinogen and also casein and gelatin. It has maximum activity and stability at a pH of 7.2

Plasminogen can readily be activated in vitro by the action of chloroform which, by destroying antiplasmin (20, 21, 39, 40, 48, 92), allows of the activation of plasminogen by some intrinsic activator system, by techniques of fractionation of the plasma (9, 17, 23, 33, 37, 48, 64, 67, 72 to 75) which separate the plasmin-antiplasmin complex and by the direct action of trypsin (41) and of a tissue activator, fibrinokinase (2, 3, 5 to 7).

Activation in vitro may also be achieved by the action of streptokinase and of staphylokinase (45). Tillet and Gardner (84) found that if human plasma clots in the presence of certain strains of beta-haemolytic streptococci rapid fibrinolysis resulted. Milstone (52) showed that fibrin formed from pure fibrinogen was resistant to the action of streptococcal culture filtrates but it lysed if a small amount of the globulin fraction of human plasma was added. This form of activation was extensively studied and anomalous results were reported. Apparently conflicting reports were reconciled largely due to the observations of Mullertz and Lassen (59) and Mullertz (57) who showed that the activation was much more complex than originally believed. It would appear that streptokinase does not act directly upon plasminogen but upon an inert 'proactivator' in plasma which is transformed to 'activator'. The activator reacts with plasminogen to form plasmin. Proactivator is not only present in plasma but it occurs in urine (8, 49,

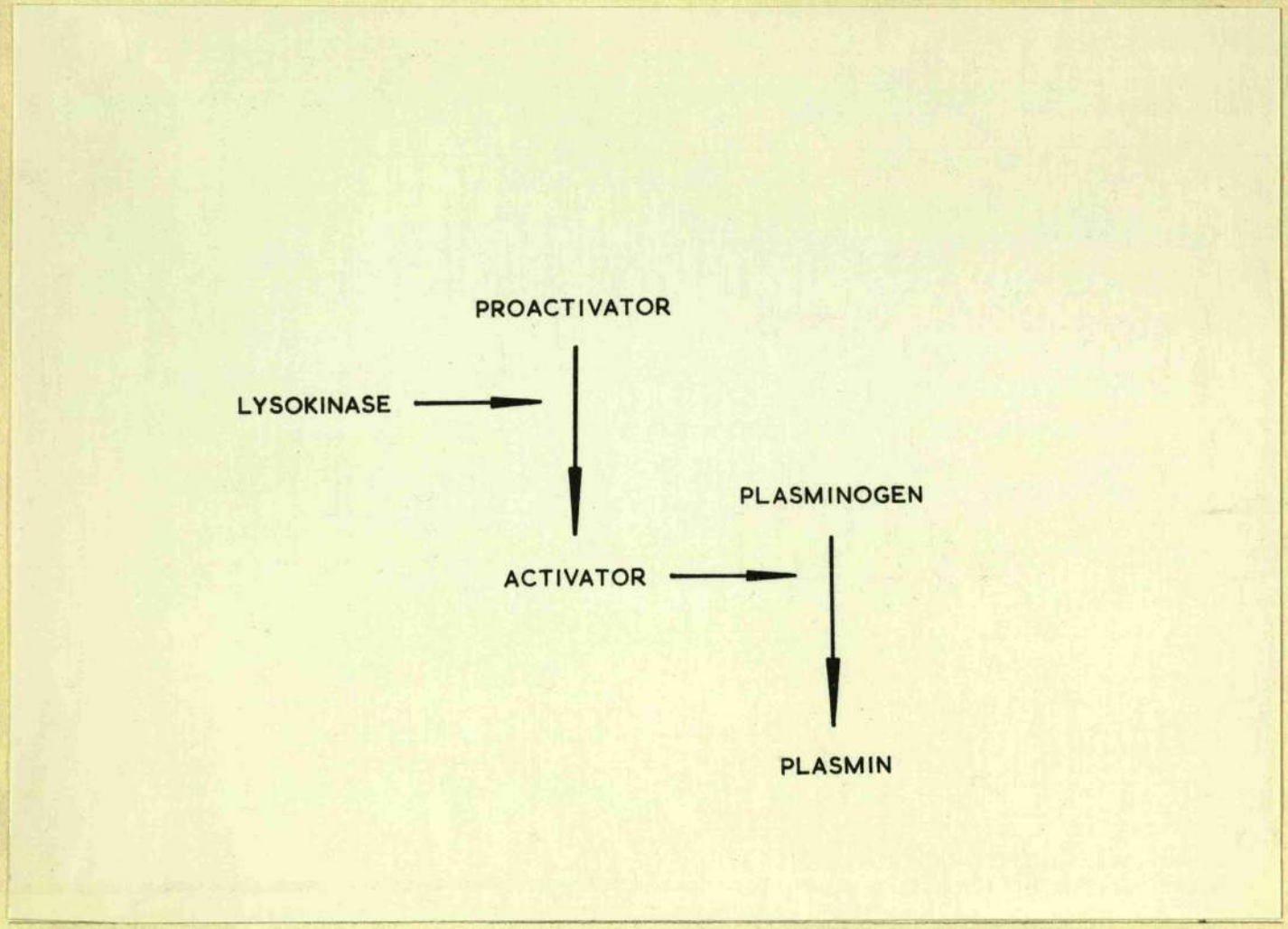

Figure 2. Simplified scheme of the activation of plasminogen in vivo

91), human milk (8A), tears (80) and saliva (1).

Figure 1 summarises the modes of activation of plasminogen to plasmin described by Astrup (4) as the "activation scheme of 1954".

Fibrinolysis in Vivo

The activation of plasminogen in vivo, that is the phenomenon referred to as spontaneous fibrinolysis, is highly complex. In simplified form proactivator is converted to activator which, following clotting, converts plasminogen to plasmin (Figure 2). Mullertz (56) first demonstrated the presence of increased amounts of activator of plasminogen but no plasmin in plasma showing spontaneous fibrinolytic activity. He (58) considered that in fluid blood the activator is bound by an inactivator and consequently no plasmin is formed. In the presence of fibrin the activator is adsorbed on to the fibrin, the effect of the inactivator is reduced, plasminogen is activated and fibrinolysis occurs.

It follows from the above that in determining the 'fibrinolytic' activity of a specimen of plasma, what is being measured indirectly is the concentration of activator present in the fluid plasma prior to clotting. The activator in fluid plasma loses activity on storage at room temperature, a process substantially reduced by storage at 0°C, (25, 27, 85).

The mode of conversion of proactivator to activator in vivo has not been established. There is evidence that there is a 'lysokinase', that is a substance capable of converting proactivator to activator, in blood and in tissue (4, 45) and it may well be that lysokinase activity is responsible for the conversion.

Induced Fibrinolytic Activity in Man

It has been shown that if a low temperature technique is employed in handling the specimen of blood (26, 28) fibrinolytic activity can be demonstrated in the plasma of healthy subjects. In the absence of this technique fibrin clots incubated at 37°C under sterile conditions remain intact for many days.

Increased fibrinolytic activity has been reported to have been induced in a variety of circumstances. Macfarlane (46) first reported its occurrence following surgical operation, a finding subsequently confirmed by the observations of Imperati (35). Macfarlane and Biggs (47) extended their observations and concluded that fear rather than the trauma of operation played the major role in the development of the fibrinolytic activity. Latner (44) reported its occurrence in normal subjects who had heard their first air raid warning. Biggs, Macfarlane and Pilling (14) showed that it could be induced in normal subjects by strenuous exercise and by the injection of adrenaline. They concluded that it was "probable that the fibrinolytic activity associated with exercise, fear and trauma follows indirectly the stimulation of adrenaline secretion". Truelove (85) found that alarming suggestions under hypnosis or anxiety in students about to take part in examinations would induce fibrinolysis. However, arguing on the variation of the eosinophil counts under these circumstances he thought it "unlikely that a high level of circulating adrenaline is the usual agent which induces fibrinolysis". According to Biggs and Macfarlane (13) Woodward found that the adrenaline levels measured immediately after severe exercise showed no correlation with the fibrinolytic activity encountered in the plasma. Berg (11) reported that there was an increase in blood phosphatide and active fibrinolysis in patients undergoing electrical convulsion therapy.

Induced Fibrinolytic Activity in Animals

Fibrinolytic activity has been induced experimentally in animals namely the dog, rabbit and rat by a variety of procedures among which have been the intravenous injection of peptone (60 to 63), anaphylactic shock (36, 71), tourniquet shock (90), post-haemorrhagic shock (81), shock following the injection of callacrein (89) and electrically induced convulsions (24). As pointed out by Biggs and Macfarlane (13) the main obstruction to the study of fibrinolytic activity experimentally

in animals is the extreme severity of the procedures required. There is one phenomenon which would appear to have been overlooked and that is the difficulty encountered in producing persistent thrombi experimentally in animals (38). Thrombi can readily be produced by a variety of techniques but commonly they rapidly lyse. The failure to produce persistent clots has been emphasised (38) but there has been a singular absence of attempt to determine the factors involved in their lysis.

Fibrinolytic Activity in Disease

Increased fibrinolytic activity of the plasma has been encountered in various pathological states. In a majority of instances patients have presented with haemorrhagic manifestations and the entity has been referred to as 'fibrinolytic purpura' (78) and as 'purpura thrombolytica' (70). Goodpasture (31) in 1914 first reported its occurrence in cirrhosis of the liver and subsequent observers (42, 50, 68, 77) have confirmed this. It has been encountered in reactions to blood transfusion (19, 30, 93), severe burns (86), haemorrhagic shock (81), metastatic carcinoma of the prostate (82, 83) and of other organs (18, 79), acute leukaemia (79), obstetrical accidents such as concealed accidental haemorrhage and amniotic embolism (22, 54, 69, 81, 88), following splenectomy for leukaemia (32) and for cryptogenetic splenomegaly (43) , following pneumonectomy or lobectomy (10, 15, 18, 51, 65, 76) and other surgical operations (16, 18, 66).

Plasma Fibrinolytic Activity in the Cadaver

According to Biggs and Macfarlane (13) the first record of the effects of post-mortem fibrinolysis was the report by Morgagni in 1769 of the finding of fluid, incoagulable blood in a man stabbed through the heart. The phenomenon was described a quarter century later by John Hunter (34): "In many modes of destroying life the blood is deprived of its power of coagulation, as happens in sudden death produced by many kinds of fits, by anger, electricity or lightning; or by a blow on the

stomach, etc. In these cases we find the blood, after death, not only in as fluid a state as in the living vessels, but it does not even coagulate when taken out of them". Virchow (87) found that the capillary blood in the cadaver was always fluid and incoagulable, and that the blood in the veins of the limbs was more often than not incoagulable. Morawitz (55) observed that there was no fibrinogen in the blood in cases of sudden death and that the blood contained a fibrinolysin. The same phenomenon was reported by Fidon, Gautier and Martin (29) in experimentally asphyxiated dogs. According to Yudin (94) Skundina and Rusakow found that the blood of individuals dying suddenly in traffic accidents, from drowning, from acute cardiac diseases and from apoplexy, though rapidly coagulating if removed within a few hours after death, became fluid again in from one-half to one and a half hours. They observed with the aid of an ultramicroscope the process of lysis and found that the fibrin network broke up into "the tiniest kernels". Mole (53) reinvestigated the problem and demonstrated the presence of a fibrinolysin in over 90% of samples of fluid and incoagulable cadaver blood, none of which contained fibrinogen. In a study of 61 autopsies he found that the blood was clotted and did not contain fibrinolysin in cases of death from infections and cachexia, whereas the reverse was true in cases of accidental and sudden death. He noted that "the more sudden the cause of death, the more likely was the blood found to be fluid". He observed that there was a centripetal decrease in the plasma fibrinolytic activity in the veins of the body and suggested that this inverse relationship between the fibrinolytic activity and the diameter of the vein from which the blood was obtained indicated that the vascular endothelial lining might be the site of production of the fibrinolysin.

Studies of postmortem fibrinolysis have also been made, according to Biggs and Macfarlane (13), by Halse on cats and rabbits killed by asphyxia or drowning and by Berg (11) on humans dying from asphyxia or haemorrhage. Both these authors found that an increase in the intensity of the

fibrinolytic activity was associated with an increase of phosphatides and of adrenaline-like substances in the blood.

It has been clearly established (12, 53) that cadaveric fibrinolysis does not differ from induced fibrinolysis in man and it has been shown that the fibrinolysis is due primarily to the appearance of activator in the blood.

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Chapter 2

MATERIAL AND METHODS

FIBRINOLYTIC ACTIVITY IN MAN

Unless otherwise stated the observations on man were made on healthy volunteer medical students and members of the staff.

Fibrinolytic Activity of the Plasma

Venous Blood

All reagents and glassware were sterile. Fibrinolytic activity was determined quantitatively by the method of Bidwell as described by Biggs and Macfarlane (2). Venous blood 4.5 ml., was withdrawn into a centrifuge tube containing 0.5 ml. of 3.8% solution of sodium citrate. The plasma was separated by centrifuging immediately after withdrawal. A series of 5 test tubes ($\frac{3}{8}$" x 3"), each containing 4.8 ml. of isotonic saline buffered to pH 7.4 with veronal acetate-hydrochloric acid buffer (9) was set up and 0.2 ml. of the plasma was added to each tube. There was added to clot the mixture 0.2 ml. of a solution of bovine thrombin, containing approximately 20 units per ml., and this, in view of the lability of activator (3, 4, 13) was done in every instance 15 minutes after withdrawal of the blood. The tubes were then incubated in a water bath at 37°C., the first tube for 30 minutes, the second, third, fourth and fifth for 3, 6, 12 and 24 hours respectively.

At the end of the incubation period the clot was removed by gently winding it around a glass rod with one end roughened for this purpose. The clot was washed three times with approximately 10 ml. ice cold normal saline allowing 15-30 minutes standing in ice between each washing. After the last washing the rod with the adherent clot was transferred to a test tube graduated to 8 ml. and 2.0 ml. of 0.1 N NaOH poured down the rod. The tube was then heated in a boiling water bath for 2 minutes. The mixture was cooled to room temperature and Na_2CO_3 solution (12.5% W/V.) added to the 8 ml. mark. One ml. of 0.01 M $CuSO_4$ was then added and, during stirring, 1.0 ml. of Folin-Ciocalteu's phenol reagent (diluted 1 in

3 with distilled water). The mixture was allowed to stand for 30 minutes to permit of full development of the blue colour and then read in a photoelectric colorimeter using a red filter against a blank prepared by using 2.0 ml. of 0.1 N NaOH and the reagents as described above. The value obtained was read directly in terms of mg. fibrin from a curve prepared by using standard quantities of tyrosine, the tyrosine fibrin conversion factor used being 10.7 (11). The initial fibrin content was taken as the amount estimated in the first tube since 30 minutes may be taken as the period of optimum conversion of fibrinogen to fibrin (6, 12).

In addition to the above, tubes were observed at hourly intervals and the time taken for total lysis to occur recorded.

Fibrinolytic Activity of the Plasma

Blood from a Finger-tip

Specimens were obtained by skin puncture and 0.2 ml. was added to a test tube containing 2.4 ml. isotonic saline buffered to pH 7.4 and 0.05 ml. of 3.8% solution of sodium citrate. The red cells were removed by centrifuging and the supernatant was clotted by the addition of 0.1 ml. of a solution of bovine thrombin containing approximately 20 units per ml. The tube was then incubated in a water bath at 37°C and inspected for 24 hours at hourly intervals for lysis.

Results

The results are recorded as the percentage of fibrin lysed per hour. It should be noted that the time-lysis relationship is not linear (1). This method of expression has been chosen for convenience in comparison of results since total lysis time was not applicable to all specimens.

Throughout this thesis in the presentation of the results of experiments and in comments thereon the term 'fibrinolytic activity of the plasma', an activity which only develops following clotting, is applied to fluid plasma. The term is employed for the sake of clarity. As stated in Chapter 1, fluid plasma, showing fibrinolytic activity following clotting,

has an increased content of activator of plasminogen but no plasmin.

Subject numbers in the tables are employed only for ease in reference to the tables.

FIBRINOLYTIC ACTIVITY IN RABBITS

Adult white rabbits were employed.

A thrombus in the marginal vein of the rabbit's ear was produced by a modification of the technique reported by Grossi, Cliffton and Cannamela (8). A length of the vein was isolated between two 'bulldog' clamps and the medial tributaries occluded by a clamp applied parallel to the marginal vein. A 25-gauge needle was inserted, the vein emptied of blood, and 0.05 ml. of a solution of bovine thrombin in normal saline, 500 units per ml., injected. The vein was allowed to fill by releasing the upper clamp which was then reapplied. After 15 minutes the clamps were removed. The segment of vein was examined at intervals by transillumination employing, if necessary, a dissecting microscope and the time taken for the clot completely to disappear determined.

Control Observations

The lysis time of a thrombus produced by the foregoing technique was found in 20 rabbits to range from 8 to 39 hours (mean = 20: S.D. = 7).

EXPERIMENTAL

The basic method employed in man was to make observations on one arm, the opposite arm serving as a control on any influence exerted by the endogenous release of adrenaline.

In the injection of substances under test alongside a vein, referred to as paravenous injection, care was taken to ensure that the point of the needle lay clear of the vein and approximately 5 mm. away from it. All substances injected were in solution in normal saline and the solution was warmed to body temperature before injection. The volume of saline employed, unless otherwise stated, was 0.1 ml. in man and 0.05 ml. in rabbits.

Thrombocytopaenia was produced in rabbits by the intravenous injection of guinea-pig anti-rabbit-platelet serum.

In view of the nature of the experiments conducted on man and on the rabbit it was necessary to determine whether a substance, capable of diffusion, when injected into a larger subcutaneous vein would pass through the wall of that vein. It has been known for many years that such a substance applied to the wall of a vein rapidly enters that vein. Muller (10) states that "Magendie laid bare one of the jugular veins in a young dog of six weeks old, and isolated it from the surrounding parts in its whole length, so that he could pass a card beneath it. He then applied freely to the vein a watery solution of spiritous extract of nux vomica. The symptoms of poisoning appeared before the fourth minute". To find out whether the converse passage occurred a thrombus was produced in the marginal vein of the ear of each of 3 rabbits by the technique described above save that the thrombin was dissolved in a 5% solution of fluorescein. The ear was examined under ultraviolet light and, within one minute of the production of the thrombus, fluorescence was detectable in the tissues surrounding the vein.

Control Observations on Man

Neither the intravenous nor paravenous injection of 0.1 ml. of a 1% solution of sodium chloride, employing the techniques described in Chapter 4, stimulated the production of fibrinolytic activity within veins. In each case observations were made on 5 subjects. One per cent saline was selected since the maximum quantity of any drug employed was 1 mg. in 0.1 ml. of isotonic saline.

Control Observations on the Rabbit

The lysis time of a thrombus produced in a marginal vein of one ear of each of 5 rabbits, by the technique described above but following the injection of 0.05 ml. of a 1% solution of sodium chloride alongside that vein, ranged from 17 to 28 hours (mean = 21; S.D. = 4) and was not

significantly different from that encountered in the untreated controls.

HISTOLOGICAL

Biopsy specimens were fixed in half strength Zenker-formalin solution. Serial sections, when cut, were cut at 7 μ. Sections were stained with Mallory's phosphotungstic acid haematoxylin, which permits of differentiation between platelets and fibrin, and with haematoxylin and eosin.

STATISTICAL METHODS

Standard statistical methods (7) have been employed throughout. By convention the probability value (P) accepted as significant is < 0.05.

OTHER METHODS

Other methods employed are more appropriately described elsewhere.

DRUGS - NOMENCLATURE

The following is a list of the abbreviations and terms used in the text for the drugs employed in the various experiments.

Drug	Text Reference
Acetylcholine bromide or chloride	Acetylcholine
Adrenaline hydrochloride	Adrenaline
Atropine sulphate	Atropine
Bovine thrombin	Thrombin
5-hydroxytryptamine creatinine sulphate	5-HT, Serotonin
Histamine acid phosphate	Histamine
L-noradrenaline bitartrate	Noradrenaline
Lysergic acid diethylamide	LSD 25
Neostigmine bromide	Neostigmine
Pituitrin (Parke Davis & Co.)	Pituitrin
Procaine hydrochloride	Procaine

Drug	Text Reference
Purified protein derivative of tuberculin	PPD
S.K.F. 42 (Fellows and Bernheim, 5)	S.K.F.42
Tolazoline	Priscol

ABBREVIATIONS

c.mm.	=	cubic millimetre
Hg.	=	mercury
kg.	=	kilogramme(s)
mg.	=	milligramme(s)
ml.	=	millilitre(s)
mm.	=	millimetre(s)
S.D.	=	standard deviation
P	=	P value (probability)
S.E.(M.)	=	standard error (of the mean)
µ	=	micron(s)
µg.	=	microgramme(s)

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Chapter 3

INDUCED PLASMA FIBRINOLYTIC ACTIVITY WITHIN VEINS IN MAN. 1. ISCHAEMIA

Chance findings in another unrelated research led to this investigation. McFadzean and Tsang (3), in the course of study of a haemorrhagic tendency encountered in 'cryptogenetic splenomegaly' (5) found that, although the positive venous pressure test was usually negative, a vascular fragility could be unmasked in a high proportion of cases if the positive venous pressure test was applied after production of reaction hyperaemia. During studies aimed at identifying the factor responsible for this unmasking of the vascular fragility, blood from the limb subjected to circulatory arrest was found to be actively fibrinolytic, a finding also encountered on investigation of normal controls. Fibrinolytic activity was investigated as a possible cause of the vascular fragility because of knowledge that increased fibrinolytic activity was responsible for other forms of haemorrhage in cryptogenetic splenomegaly (2).

A review of the literature revealed two relevant communications. Tagnon, Levenson, Davidson and Taylor (6) observed the occurrence of increased fibrinolytic activity in patients with peripheral circulatory failure following haemorrhage or severe burns and showed, in dogs, that such activity followed the production of haemorrhagic shock. They considered the fibrinolytic activity was the result of a prolonged anoxic state consequent upon peripheral circulatory failure. On the other hand Biggs and Macfarlane (1) stated that anoxia of a limb produced by a tourniquet does not lead to fibrinolytic activity in the distal part of a limb. The technique employed was not described but, as will be seen later, it is of importance to record that in a personal communication Macfarlane (4) stated that in their experiments each of the subjects exercised the muscles of the forearm during the phase of arrest of the arterial circulation.

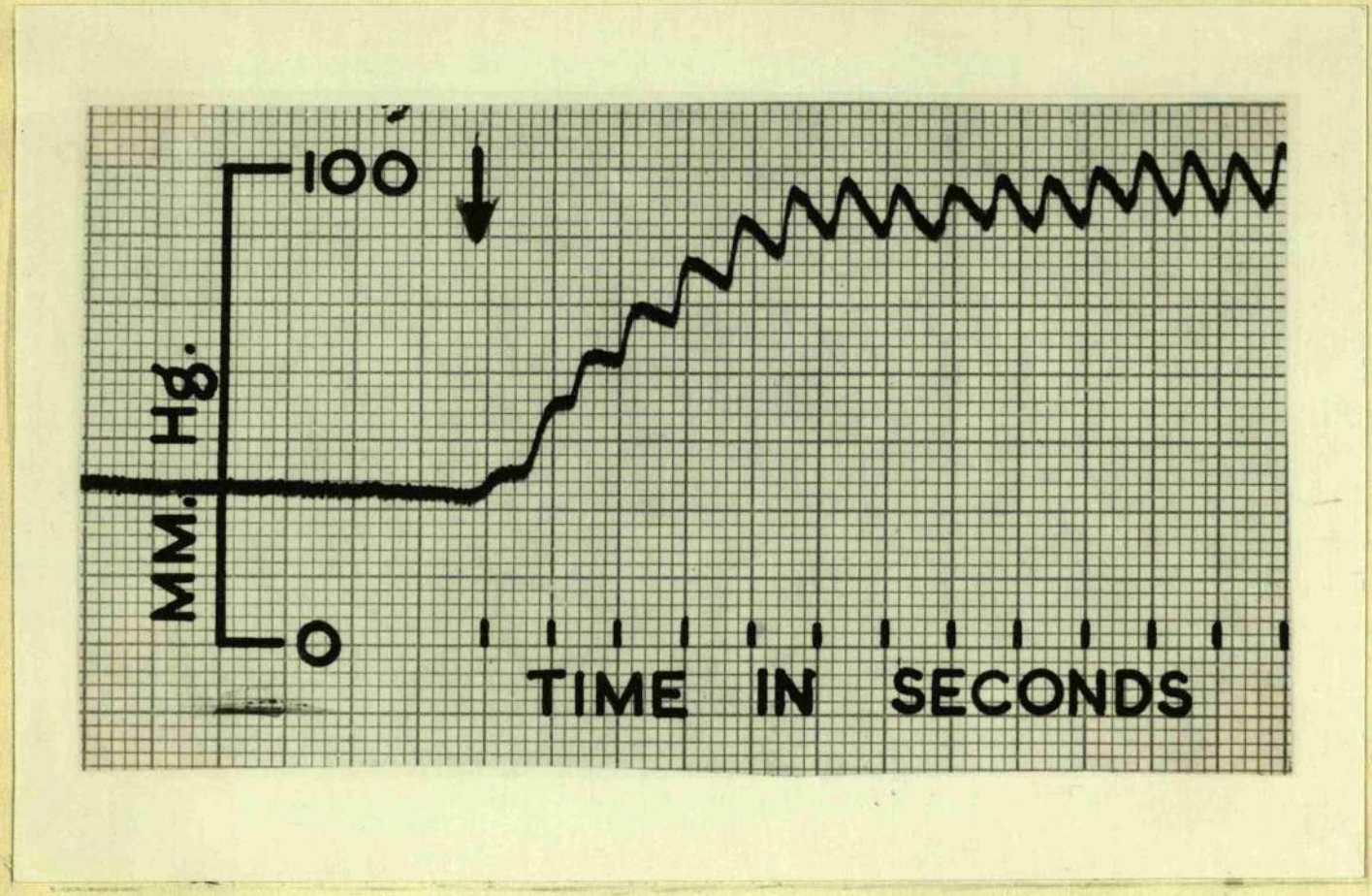

Figure 3. The rise in pressure in an antecubital vein following reduction in pressure in a sphygmomanometer cuff (arrow) applied above the elbow, from above the systolic pressure, which had been maintained for 10 minutes, to the diastolic pressure.

EXPERIMENTS

Preliminary Observations

In view of the experiments subsequently to be described it was necessary to determine the time taken for the pressure in an antecubital vein following a period of arrest of the arterial circulation to rise to that of a sphygmomanometer cuff applied above the elbow and inflated to the diastolic pressure. Observations were made on 6 subjects. A needle connected to an electromanometer and recording apparatus was inserted into an antecubital vein and a sphygmomanometer cuff applied above the elbow was inflated to 20 mm. Hg. above the systolic blood pressure. Ten minutes later the pressure in the cuff was reduced to the diastolic level.

The time taken for the venous pressure to rise above the diastolic level ranged from 3 to 5 seconds. Figure 3 is a typical record.

Experiment 1

This was undertaken to determine if arrest of the arterial supply to part of a limb resulted in fibrinolytic activity occurring in the plasma of blood subsequently flowing through that part.

Observations were made on 20 subjects. A sphygmomanometer cuff applied to one arm above the elbow was inflated to 20 mm. Hg. above the systolic blood pressure and maintained for 10 minutes. The pressure was then reduced to the diastolic level. Five minutes later specimens of blood were obtained from an antecubital vein of both the experimental and control arms and the fibrinolytic activity of each specimen determined.

The results are set out in Table I. It will be seen that the fibrinolytic activity of each specimen from the experimental arm was significantly greater than that of the corresponding specimen from the control arm.

Table I

Plasma fibrinolytic activity in experimental and control arms in Experiment 1

Subject No.	Plasma fibrinolytic activity (Percentage of fibrin lysed per hour)	
	Experimental arm	Control arm
1	8.3	0.7
2	1.9	0.4
3	4.1	0.3
4	18.7	0.2
5	33.0	0.9
6	16.6	0.9
7	33.0	0.6
8	8.3	0.6
9	33.0	0.2
10	1.9	0.2
11	2.2	0.8
12	2.2	0.3
13	6.1	0.8
14	33.0	0.2
15	1.1	0.2
16	33.0	0.7
17	16.6	0.1
18	33.0	0.3
19	16.6	0.3
20	11.2	1.0
Mean (S.D.)	14.2 (12.1)	0.5 (0.3)

Experiment 2

This was undertaken to determine whether venous occlusion alone would result in the development of fibrinolytic activity in the plasma.

Observations were made on 10 subjects. A sphygmomanometer cuff was applied to one arm above the elbow and inflated to the diastolic pressure. This was maintained for 10 minutes in 5 subjects (Group 1) and for 30 minutes in the remaining 5 (Group 2). At the end of these times specimens of blood were obtained from an antecubital vein in the experimental arm and in the control arm and the fibrinolytic activity of each specimen determined.

Table II

Plasma fibrinolytic activity in experimental and control arms in Experiment 2

Subjects	Plasma fibrinolytic activity (Percentage of fibrin lysed per hour)	
	Experimental arm	Control arm
Group 1	Mean = 0.5 S.D. = 0.3	Mean = 0.3 S.D. = 0.2
Group 2	Mean = 0.3 S.D. = 0.2	Mean = 0.4 S.D. = 0.2

The results are set out in Table II. In neither Group was there a significant difference between the fibrinolytic activity of the plasma from the experimental limb and that from the control limb.

Experiment 3

This was undertaken to determine if, following ischaemia, fibrinolytic activity was present in the plasma of blood flowing through the capillary bed.

Observations were made on 10 subjects. Experiment 1 was repeated but in addition to the venous samples specimens of blood were obtained from a finger-tip in both the experimental and control limbs.

Table III

Plasma fibrinolytic activity in experimental and control arms in Experiment 3

Subject No.	Plasma fibrinolytic activity (Percentage of fibrin lysed per hour)			
	Experimental arm		Control arm	
	Venous blood	Finger-tip blood	Venous blood	Finger-tip blood
1	8.3	8.3	0.7	None lysed in 24 hours
2	1.9	5.5	0.4	
3	33.0	33.0	0.2	
4	6.1	6.6	0.8	
5	2.2	8.3	0.8	
6	16.6	16.6	0.9	
7	1.1	5.1	0.2	
8	8.3	16.6	0.6	
9	18.7	16.6	0.2	
10	4.1	6.6	0.3	
Mean (S.D.)	10.0 (9.5)	12.3 (8.2)	0.5 (0.3)	-

The results are set out in Table III. In all cases the fibrinolytic activity of both specimens from the experimental arm was greater than that of the control specimens. Comparison of the two specimens from the experimental arm shows the fibrinolytic activity of blood from the finger-tip was greater than that of the venous blood in 5 instances, equal to it in 4 and less in the remaining one instance.

Experiment 4

This was undertaken to determine whether fibrinolytic activity was imparted to blood within veins when the arterial supply was arrested and, if so, to compare it in intensity with that encountered in venous blood and blood from the finger-tip subsequent to restoration of the circulation.

Observations were made on 5 subjects. A sphygmomanometer cuff was

applied above the elbow and inflated to the diastolic pressure to allow the veins to fill. Once the superficial veins were distended the pressure in the cuff was raised to 20 mm. Hg. above the systolic pressure and maintained for 10 minutes when a sample of blood was obtained from an antecubital vein in the experimental arm (Spec.1, Table IV) and in the control arm. The pressure in the cuff was then reduced to the diastolic level. Specimens of blood were then obtained from another antecubital vein in the experimental arm (Spec.2, Table IV) and from a finger-tip in both the experimental and control arms.

Table IV

Plasma fibrinolytic activity in experimental and control arms in Experiment 4

Subject No.	Plasma fibrinolytic activity (Percentage of fibrin lysed per hour)				
	Experimental arm			Control arm	
	Spec.1	Spec.2	Finger-tip	Venous blood	Finger-tip blood
1	2.7	5.7	16.6	0.3	None lysed in 24 hours
2	2.0	4.8	8.3	0.6	
3	2.6	10.4	16.6	0.4	
4	8.3	14.3	16.6	0.6	
5	2.3	4.1	8.3	0.3	

From Table IV it will be seen that in each case the fibrinolytic activity of the plasma of all specimens from the experimental arm was significantly greater than that from the control arm. In each case the fibrinolytic activity of Specimen 2 was greater than that of Specimen 1 and further the fibrinolytic activity of blood from the finger-tip was greater than that of Specimen 2.

Experiment 5

This was undertaken to determine whether there was a centrifugal increase in fibrinolytic activity in the veins, a possibility suggested by the results of Experiments 3 and 4.

Table V

Plasma fibrinolytic activity in successive specimens removed from the same vein in Experiment 5

Subject No.	Plasma fibrinolytic activity (Percentage of fibrin lysed per hour)				
	Specimen				
	1	2	3	4	5
1	3.6	3.6	4.1	6.9	8.3
2	2.2	1.9	4.3	6.2	-

Observations were made on 2 subjects. A cuff was applied above the elbow and inflated to the diastolic level. Once the superficial veins were distended the pressure in the cuff was raised to 20 mm. Hg. above the systolic blood pressure and maintained for 10 minutes. Venepuncture was then performed and 5 consecutive specimens of 5 ml. each were removed as rapidly as possible through the same needle. The fibrinolytic activity of each specimen was determined.

The results are set out in Table V from which it will be seen that there was a progressive increase in fibrinolytic activity in each successive specimen in both cases following the second specimen.

Experiment 6

It seemed desirable to determine whether arrest of the circulation to a finger alone would result in the production of increased fibrinolytic activity in blood contained therein and if so how long this increased activity persisted following restoration of the circulation.

Observations were made on 4 subjects. Venous return from a finger was impeded by a ligature until the veins were distended. The ligature was then tightened to occlude the arterial supply. Ten minutes later a specimen of blood was obtained from the finger-tip (Spec.1, Table VI) and from the tip of another finger of the same hand (Control finger-tip, Table VI). The ligature was released and a specimen obtained one minute later (Spec.2, Table VI).

Table VI

Plasma fibrinolytic activity in experimental and control arms in Experiment 6

Subject No.	Plasma fibrinolytic activity (Percentage of fibrin lysed per hour)		
	Experimental arm		Control finger-tip
	Spec.1 (Finger-tip)	Spec.2 (Finger-tip)	
1	8.3	None lysed in 24 hours	None lysed in 24 hours
2	16.7		
3	12.5		
4	12.5		

In all cases Specimen 1 showed significant fibrinolytic activity. In each case Specimen 2 and the control failed to lyse in 24 hours.

Experiment 7

The results of Experiment 6 rendered it necessary to determine the source of the increased activity encountered in blood from the finger-tip 5 minutes after restoration of the circulation in Experiment 3. Either this was locally produced in the small vessels of the finger-tip or alternatively it had its origin in the arterial tree in the ischaemic part of the limb.

Experiment 1 was repeated on 4 subjects and 5 minutes after reduction of the pressure to the diastolic level specimens were obtained from the radial artery (Spec.1, Table VII), from an antecubital vein (Spec.2, Table VII), and from an antecubital vein in the control arm. It should be noted that local procaine anaesthesia was used prior to obtaining the specimen from the radial artery.

Table VII

Plasma fibrinolytic activity in experimental and control arms in Experiment 7

Subject No.	Plasma fibrinolytic activity (Percentage of fibrin lysed per hour)		
	Experimental arm		Control arm (Antecubital vein)
	Spec.1 (Radial artery)	Spec.2 (Antecubital vein)	
1	5	16.6	5
2	16.6	14.2	8.2
3	12.5	6.6	6.6
4	16.6	11.1	5.5

In 3 cases the fibrinolytic activity of Specimen 1 was greater than that of Specimen 2 or of the control. In the remaining case it was less than that of Specimen 2 and equal to the control. The fibrinolytic activity of each of the control specimens was considerably increased.

Experiment 8

This was undertaken to determine whether ischaemia would stimulate the production of fibrinolytic activity within veins distal in the line of flow to the occluding cuff.

Observations were made on 4 subjects. Figure 4 illustrates this experiment. A sphygmomanometer cuff was applied above the elbow (the

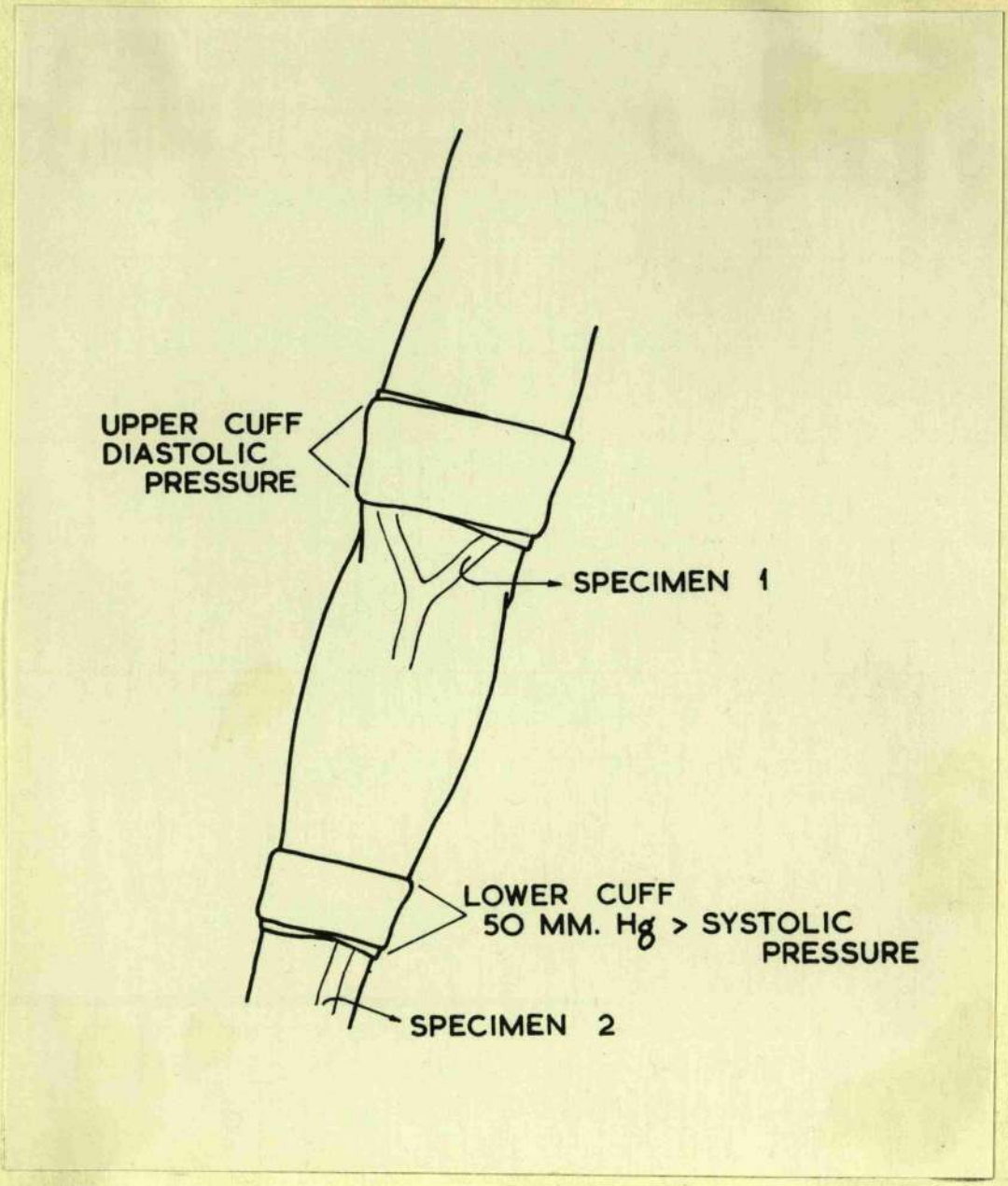

Figure 4. Experiment 8

upper cuff) and a second cuff over the mid-forearm. The upper cuff was inflated to the diastolic pressure and the lower cuff, once the veins were distended, was inflated to 50 mm. Hg. above the systolic pressure.

Table VIII

Plasma fibrinolytic activity in experimental and control arms in Experiment 8

Subject No.	Plasma fibrinolytic activity (Percentage of fibrin lysed per hour)		
	Experimental arm		Control arm
	Spec. 1	Spec. 2	
1	11.1	7.7	0.2
2	12.5	8.3	0.3
3	10.0	5.2	0.5
4	8.3	4.7	0.2

Samples of blood were removed 5 minutes later from an antecubital vein between the cuffs (Spec.1, Table VIII), from a vein at the wrist below the lower cuff (Spec.2, Table VIII), and from an antecubital vein of the control arm.

While fibrinolytic activity developed in both Specimens 1 and 2, in all 4 subjects that of Specimen 1 was significantly greater than that of Specimen 2.

Comment

It has been shown that arrest of the arterial supply to part of a limb results in the development of fibrinolytic activity in blood in veins within that part. The intensity of this activity is significantly less than that encountered in blood flowing through the limb 5 minutes subsequent to restoration of the arterial flow. It can be taken that

this latter activity is imparted to the blood in course of circulation through the part of the limb previously rendered ischaemic. The finding in Experiments 3 and 4 that the fibrinolytic activity of 'capillary' blood is greater than that of blood from an antecubital vein is consistent with the major activity developing either in the 'capillary' bed or in the arterial tree within that part of the limb rendered ischaemic. The demonstration in the ischaemic limb, Experiment 5, of a centrifugal increase in fibrinolytic activity in the venous tree would appear to support the former as the source. However the results of Experiment 6 are against this. In this experiment it has been shown that if a finger is rendered ischaemic the fibrinolytic activity which develops in 'capillary' blood disappears within one minute of restoration of the arterial flow. Experiment 7 would appear to establish the arterial tree as the site of the major development of fibrinolytic activity. It will be seen, Chapter 8, that this evidence is suspect since it is shown therein that procaine stimulates the production of fibrinolytic activity within an artery. Nevertheless the evidence is in favour of the site of the major development of fibrinolytic activity following restoration of arterial flow in an ischaemic limb being the arterial tree.

The finding in Experiment 5 of a centrifugal increase in fibrinolytic activity within veins during circulatory arrest is consistent with the activity being imparted by the vessel wall.

It has been shown that arrest of the arterial circulation to the distal part of an arm by an inflated cuff encircling the limb not only results in the development of fibrinolytic activity in the plasma of blood within veins in the part rendered ischaemic but it also results in fibrinolytic activity of even greater intensity, developing within veins in the proximal part of the limb above the cuff. This suggests the possibility of a reflex mechanism being involved.

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Chapter 4

INDUCED PLASMA FIBRINOLYTIC ACTIVITY WITHIN VEINS IN MAN

2. ADRENALINE, ACETYLCHOLINE, HISTAMINE, PITUITRIN

Since it has been shown, Chapter 3, that plasma fibrinolytic activity develops within veins in a limb rendered ischaemic it seemed possible that subcutaneous veins of the arm might be employed in an investigation of the effect of other stimuli.

Initial observations were with adrenaline since this was the only substance known to induce fibrinolysis in man. Biggs, Macfarlane and Pilling (1) first showed that the subcutaneous injection of adrenaline induced fibrinolytic activity of the plasma in normal subjects.

EXPERIMENTS

Preliminary Observations

In view of the nature of the experiments subsequently to be described it was necessary to determine the time taken for the pressure in an antecubital vein to rise to that of a sphygmomanometer cuff applied above the elbow and inflated to the diastolic pressure. Observations were made on 8 subjects. A needle connected to an electromanometer and recording apparatus was inserted into an antecubital vein and a sphygmomanometer cuff applied above the elbow was inflated to the diastolic pressure.

The time taken for the venous pressure to rise to that in the cuff ranged from 165 to 210 (mean 188 with S.E. $\pm$ 6) seconds.

ADRENALINE

Experiment 9A

Observations were made on 3 subjects. Adrenaline hydrochloride, 1 µg. in 0.1 ml. normal saline, was injected into the median basilic vein at the elbow. Five minutes later a sphygmomanometer cuff was applied above the elbow and a specimen of blood taken from the vein at the site of the injection. At the same time a specimen of venous blood was obtained from the opposite arm.

The fibrinolytic activity of the plasma of each of the specimens was within normal limits and in no case was there significant difference between the activity of plasma from the experimental limb and that of the control.

Experiment 9B

This was undertaken to determine if a period of retention of adrenaline within a segment of a vein would result in the development of fibrinolytic activity in the plasma of blood within that vein.

Observations were made on 5 subjects. A sphygmomanometer cuff was applied above the elbow of the experimental arm and inflated to the diastolic pressure. Adrenaline hydrochloride, 1 µg. in 0.1 ml. of normal saline, was injected into the median basilic vein in the antecubital fossa and 5 minutes later samples of blood were obtained from that vein (Spec.1, Table IX), from a vein at the wrist (Spec.2, Table IX) and from an antecubital vein of the control arm.

Table IX

Plasma fibrinolytic activity in experimental and control arms following the injection of 1 µg. of adrenaline into the median basilic vein in Experiment 9B

Subject No.	Plasma fibrinolytic activity (Percentage of fibrin lysed per hour)		
	Experimental arm		Control arm (Antecubital vein)
	Spec.1 (Median basilic vein)	Spec.2 (Wrist vein)	
1	8.3	3	0.3
2	6.7	6.7	4.2
3	8.3	8.3	2.7
4	8.3	6.7	2
5	4.2	2.1	0.6

In each case the fibrinolytic activity of Specimens 1 and 2 were significantly greater than that of the control. If the fibrinolytic activity of Specimens 1 and 2 are compared it will be seen that equally intense activity had developed in both specimens in 2 subjects.

Experiment 10A

Observations were made on 3 subjects. Adrenaline hydrochloride 10 µg. in 0.1 ml of normal saline was injected alongside the median basilic vein in the antecubital fossa. Five minutes later a sphygmomanometer cuff, applied above the elbow, was inflated to the diastolic pressure and a specimen of blood was removed from the segment of vein alongside which the adrenaline had been injected. A specimen of venous blood was removed from the corresponding vein of the opposite arm.

The fibrinolytic activity of the plasma of each of the specimens was within normal limits and in no case was there significant difference between the activity of plasma from the experimental limb and that of the control.

Experiment 10B

This was undertaken to determine if the presence of an inflated cuff around the arm above the site of paravenous injection of adrenaline would result in the development of fibrinolytic activity in the plasma of blood within the vein.

Observations were made on 21 subjects. A sphygmomanometer cuff was applied above the elbow on the experimental arm and inflated to the diastolic pressure. Adrenaline hydrochloride, 10 µg. in 0.1 ml. of normal saline, was injected alongside the median basilic vein of the experimental arm as in Experiment 10A. Five minutes later samples of blood were obtained from that vein (Spec.1, Table X), from a vein at the wrist (Spec.2, Table X) and in 7 subjects, from the tip of the middle finger (Spec.3, Table X). A sample of venous blood was also taken in each subject from the control arm.

Table X

Plasma fibrinolytic activity in experimental and control arms following the injection of 10 µg. of adrenaline alongside the median basilic vein in Experiment 10B

Subject No.	Plasma fibrinolytic activity (Percentage of fibrin lysed per hour)			
	Experimental arm			Control arm (Antecubital vein)
	Spec.1 (Median basilic vein)	Spec.2 (Wrist vein)	Spec.3 (Finger-tip)	
1	12.5	12.5	-	3.0
2	10.0	10.0	-	4.2
3	6.7	6.7	-	0.3
4	6.7	6.7	-	0.7
5	5.0	5.0	-	0.6
6	6.7	6.7	-	2.5
7	5.0	4.6	-	1.4
8	14.3	11.1	-	5.5
9	6.3	6.3	-	2.5
10	6.3	6.3	-	2.7
11	16.6	16.6	-	5.5
12	12.5	12.5	-	6.3
13	14.3	8.3	-	0.6
14	12.5	12.5	-	3.0
15	8.3	4.2	5.9	4.0
16	20.0	5.0	4.3	4.2
17	14.3	14.3	8.3	5.9
18	6.7	6.7	4.6	4.6
19	16.6	16.6	5.9	5.9
20	12.5	12.5	16.6	8.3
21	12.5	11.1	8.3	2.0

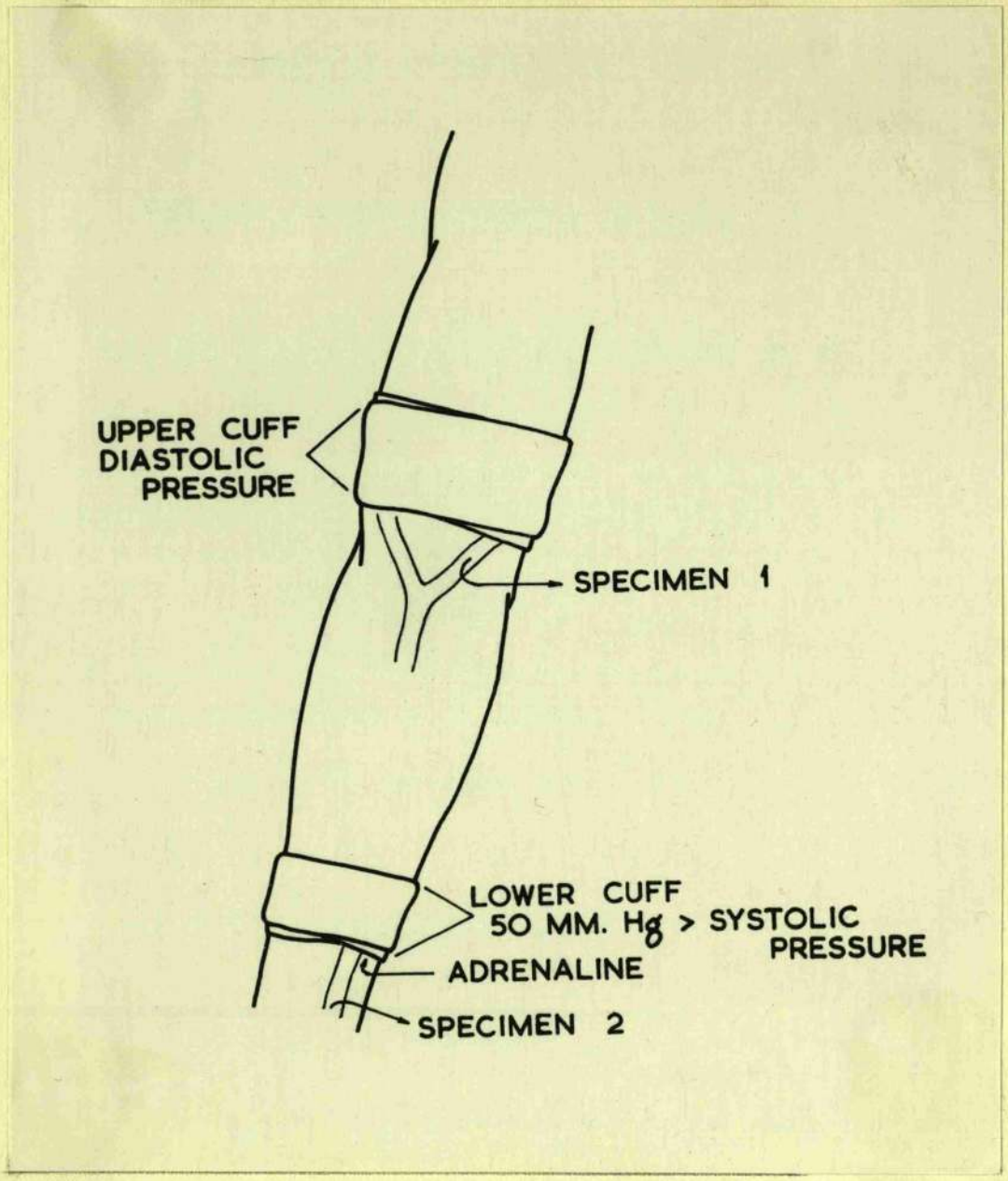

Figure 5. Experiment 11

In each case the fibrinolytic activity of Specimen 1 from the vein near the site of injection was significantly greater than that of the control and in all save Nos.15 and 16; this was also true of Specimen 2 obtained from a wrist vein. In the two exceptions there was no significant difference. The fibrinolytic activity of capillary blood (Spec.3) in 4 cases was significantly greater than that of the control but in the remaining 3 there was no significant difference. If the fibrinolytic activity of Specimens 1 and 2 are compared it will be seen that in 16 of the 21 subjects equally intense activity had developed in both specimens.

This experiment was repeated on other subjects with progressively smaller doses of adrenaline and the specimens of blood have been taken at shorter intervals following the injection. The smallest dose of adrenaline employed was 1 µg. in 0.1 ml. of saline and this had an effect equal to that reported for 10 µg. Fibrinolytic activity has been found to develop within 2 minutes of the injection and it was even more intense than that after 5 minutes.

Experiment 11

In both Experiments 9B and 10B the development of fibrinolytic activity in veins, well removed proximally in the line of flow from the site of injection, suggested that a reflex mechanism was involved.

Observations were made on 4 subjects. Figure 5 illustrates this experiment. A sphygmomanometer cuff was applied above the elbow (the upper cuff) and a second cuff over the mid-forearm (the lower cuff). The upper cuff was inflated to the diastolic pressure and the lower cuff, once the veins were distended, was inflated to 50 mm. Hg. above the systolic pressure. Adrenaline, 10 µg. in 0.1 ml. of saline was injected alongside a vein below the lower cuff. Samples of blood were removed 5 minutes after the injection from an antecubital vein between the cuffs (Spec.1, Table XI), from the vein at the site of injection (Spec.2, Table XI) and from an antecubital vein of the opposite arm. The fibrinolytic activity of the plasma of each specimen was estimated.

Table XI

Plasma fibrinolytic activity in experimental and control arms in Experiment 11

Subject No.	Plasma fibrinolytic activity (Percentage of fibrin lysed per hour)		
	Experimental arm		Control arm
	Spec.1	Spec.2	
1	16.6	4.6	4.3
2	16.6	4.6	1.0
3	11.1	7.7	0.2
4	12.5	8.3	0.3

Fibrinolytic activity developed in both Specimens 1 and 2 in all 4 subjects but that of Specimen 1 was significantly greater than that of Specimen 2. On comparison of these results with those obtained with ischaemia alone (Table VIII, Chapter 3) no significant difference emerges. Adrenaline failed to induce fibrinolytic activity greater than that which would have resulted from ischaemia alone.

The experiment has been repeated with the adrenaline injected into the vein. The response did not differ from that reported for the paravenous injection.

ACETYLCHOLINE

Experiment 12

Experiment 9 was repeated on 3 subjects substituting for adrenaline 100 µg. of acetylcholine in 0.1 ml. of saline.

The results are set out in Table XII. In each case the fibrinolytic activity of Specimen 1 was greater than that of Specimen 2 which in turn

Table XII

Plasma fibrinolytic activity in experimental and control arms following the injection of 100 µg. of acetylcholine into the median basilic vein in Experiment 12

Subject No.	Plasma fibrinolytic activity (Percentage of fibrin lysed per hour)		
	Experimental arm		Control arm (Antecubital vein)
	Spec.1 (Median basilic vein)	Spec.2 (Wrist vein)	
1	33.3	16.6	6.6
2	7.2	5.5	2.0
3	20.0	16.6	9.0

was significantly greater than that of the control.

Experiment 13

Experiment 10B was repeated on 11 subjects substituting for adrenaline the following quantities of acetylcholine in 0.1 ml. saline: 1 mg. (3 subjects); 10 µg. (6 subjects); 1 µg. (2 subjects).

The results are set out in Table XIII. No effect was exerted by 1 µg. With a dosage of 10 µg. and of 1 mg. the fibrinolytic activity of Specimen 1 and of Specimen 2 in each case were significantly greater than that of the control. If the fibrinolytic activity of Specimens 1 and 2 are compared, in 6 of the 9 subjects equally intense activity was encountered.

Table XIII

Plasma fibrinolytic activity in experimental and control arms following the paravenous injection of acetylcholine in Experiment 13

Subject No.	Quantity of acetylcholine in 0.1 ml. saline	Plasma fibrinolytic activity (Percentage of fibrin lysed per hour)		
		Experimental arm		Control arm (Antecubital vein)
		Spec.1 (Median basilic vein)	Spec.2 (Wrist vein)	
1	1 mg.	12.5	12.5	6.7
2	1 mg.	11.1	11.1	4.2
3	1 mg.	25.0	22.0	6.7
4	10 µg.	25.0	14.3	11.1
5	10 µg.	16.6	16.6	10.0
6	10 µg.	6.0	6.0	4.0
7	10 µg.	16.6	12.5	6.2
8	10 µg.	14.3	14.3	6.2
9	10 µg.	16.6	16.6	9.0
10	1 µg.	1.1	1.1	1.1
11	1 µg.	0.6	0.8	0.4

Experiment 14

Experiment 11 was repeated on 4 subjects substituting for adrenaline 10 µg. of acetylcholine in 0.1 ml. saline. Paravenous injection was employed in 2 and intravenous injections in the remainder.

Table XIV

Plasma fibrinolytic activity in experimental and control arms in Experiment 14

Subject No.	Plasma fibrinolytic activity (Percentage of fibrin lysed per hour)		
	Experimental arm		Control arm
	Spec.1	Spec.2	
1	11.1	6.6	2.1
2	14.2	6.6	3.6
3	9.0	5.8	3.2
4	10.4	6.2	1.7

The results are set out in Table XIV and are similar to those reported in Experiment 11. On comparison of these results with those obtained with ischaemia alone (Table VIII, Chapter 3) no significant difference emerges. Acetylcholine failed to induce fibrinolytic activity greater than that which would result from ischaemia alone.

HISTAMINE

Experiment 15

Observations were made on 12 subjects who were divided into two equal groups. A sphygmomanometer cuff was applied above the elbow of the experimental arm and inflated to the diastolic pressure. Histamine, 0.25 µg. in 0.1 ml. of normal saline was injected in one group into an antecubital vein and in the other alongside an antecubital vein. Five minutes later samples of blood were obtained from that vein and from an antecubital vein of the control arm and the fibrinolytic activity of each sample determined.

The results are set out in Table XV. With the exception of subjects No. 6 and 12, in each case the fibrinolytic activity of the specimen from the experimental arm was significantly increased and was greater than that from the control arm. The activity of the specimen from the experimental

Table XV

Plasma fibrinolytic activity in experimental and control arms following the injection of 0.25 µg. of histamine in 0.1 ml. of normal saline into and alongside an antecubital vein in Experiment 15

Subject No.	Route of administration	Plasma fibrinolytic activity (Percentage of fibrin lysed per hour)	
		Experimental arm	Control arm
1	Intravenous	16.6	1.1
2	"	16.6	8.3
3	"	20.0	4.1
4	"	20.0	4.1
5	"	25.0	4.1
6	"	1.4	0.1
7	Paravenous	20.0	10.0
8	"	20.0	1.2
9	"	28.5	5.2
10	"	12.5	4.1
11	"	20.0	1.2
12	"	2.5	1.1

arm in the 2 exceptions, one in each group, although greater than that from the control arm was not significantly increased. There was no significant difference between the fibrinolytic activity resulting from the paravenous injection of histamine and that from the intravenous injection.

Experiment 16

This experiment was undertaken to determine whether the intradermal injection of histamine would provoke fibrinolytic activity within veins. If such did occur it would perhaps allow not only of more ready investi-

gations of the affectors but it would permit investigation of the effects of inflammation.

Observations were made on 7 subjects. A sphygmomanometer cuff was applied above the elbow of the experimental arm and inflated to the diastolic pressure. Histamine, 0.25 µg. in 0.05 ml. of saline, was injected intradermally in 4 subjects on the volar aspect of the forearm

Table XVI

Plasma fibrinolytic activity in experimental and control arms following the intradermal and subcutaneous injection of histamine in Experiment 16

Subject No.	Route of administration	Plasma fibrinolytic activity (Percentage of fibrin lysed per hour)		
		Experimental arm		Control arm
		Spec.1	Spec.2	
1	Intradermal	2.0	1.4	0.9
2	"	1.6	1.2	0.6
3	"	5.0	4.2	2.1
4	"	2.6	1.3	0.2
5	Subcutaneous	3.8	3.3	1.2
6	"	4.7	3.0	1.1
7	"	16.6	6.6	4.0

approximately mid-way between the wrist and elbow. In the remaining 3 subjects the histamine was injected subcutaneously in the mid-forearm in an area free from visible veins. Five minutes later specimens of blood were obtained from an antecubital vein (Spec.1, Table XVI), from a vein at the wrist (Spec.2, Table XVI) and from an antecubital vein in the control arm.

In each case the activity of Specimens 1 and 2 were greater than that of the control. With one exception the activity of Specimen 1 was

greater than that of Specimen 2. The subcutaneous injection was a more potent stimulus than intradermal injection but both fell short of the intensity encountered following intravenous or paravenous injection.

For other experiments the writer had had the lateral cutaneous nerve of his left forearm cut at the point of emergence through the deep fascia lateral to the tendon of the biceps. In the area of anaesthesia the flare response to the intradermal injection of histamine was absent. Experiment 16 was repeated, the histamine being injected intradermally into the anaesthetic area. The fibrinolytic activity of Specimen 1, 2 and of that from the control arm was 0.3, 0.2, and 0.3% lysed per hour respectively.

PITUITRIN

Experiment 17

Experiment 9B was repeated on 3 subjects substituting for adrenaline Pituitrin, 0.05 ml. of a solution containing 1 unit per ml.

The results are set out in Table XVII. The fibrinolytic activity of Specimen 1 was in each case greater than that of Specimen 2 which in turn was greater than that from the control arm.

Table XVII

Plasma fibrinolytic activity in experimental and control arms following the injection of Pituitrin into (Experiment 17) and alongside (Experiment 18) an antecubital vein

Subject No.	Route of administration	Plasma fibrinolytic activity (Percentage of fibrin lysed per hour)		
		Experimental arm		Control arm
		Spec.1	Spec.2	
1	Intravenous	20.0	9.0	6.2
2	"	14.2	5.8	3.7
3	"	25.0	6.2	0.9
4	Paravenous	20.0	6.2	5.5
5	"	25.0	9.0	4.3
6	"	33.0	10.0	9.0

Experiment 18

Experiment 10B was repeated on 3 subjects substituting for adrenaline, Pituitrin, 0.05 ml. of a solution containing 1 unit per ml.

The results are set out in Table XVII. The fibrinolytic activity of Specimen 1 was in each case greater than that of Specimen 2 which was in turn greater than that from the control arm.

Comment

The observations establish that, under the conditions of the experiments, the intravenous or paravenous injection of adrenaline, acetylcholine, histamine or pituitrin results in the development of fibrinolytic activity in the plasma of blood within that vein. The increased fibrinolytic activity was not restricted to the segment of vein into or in the vicinity of which the injection was given for it was also encountered in the blood obtained from veins well removed proximally in the line of venous flow from the site of injection. It should be noted that there was evidence, Experiment 10B, of increased activity in 'capillary' blood from the finger-tip.

In the observations on fibrinolytic activity induced by ischaemia, described in Chapter 3, the mean fibrinolytic activity encountered in the plasma of blood from the control limbs in 35 subjects was 0.5 (S.E. $\pm$ 0.1) % lysed per hour and this was not significantly different from that encountered in healthy controls in the absence of experimental observations. In the large majority of the observations reported in this chapter when fibrinolytic activity was induced in the experimental limb by paravenous or intravenous injection that encountered in the control limb was significantly higher than in healthy resting subjects. For example, the mean fibrinolytic activity of the plasma from the control limbs in the 21 subjects of Experiment 10B was 3.5 (S.E. $\pm$ 0.5) % lysed per hour and in the 14 subjects in Experiments12 and 13 it was 4.7 $\pm$ 0.5. While the procedures employed may well have induced anxiety, many of the subjects were

seasoned to such experiments and had never before shown this reaction. It might well be that fibrinolytic activity was being stimulated in veins of the control limb by the intravenous or paravenous injections into the experimental limb.

It has been shown, Chapter 3, that venous occlusion alone does not induce fibrinolytic activity. In the present experiments its employment not only allows the retention of the agent injected for a significant period of time within the segment of vein in the case of intravenous injection but it also arrests the flow of blood through that segment for the same time. This latter effect presumably is the only significant effect in the case of the paravenous injection of vasoconstrictor substances. However, since specimens were removed 5 minutes after the injection of the drugs under test, that is 1½ minutes after the maximum time required for venous pressure to rise above that of the occluding cuff (Preliminary Observations), it may be stated that flow under the cuff had been reestablished and the local effect of the drug employed was not being adequately determined.

The failure of adrenaline and acetylcholine, when injected alongside a vein in a part of limb rendered ischaemic, to induce fibrinolytic activity greater than that which would result from ischaemia alone suggests that for maximal activity to develop the circulation through the limb is required.

The absence of response to the intradermal injection of histamine when such is injected into an area deprived of its sensory nerve supply although it is a solitary finding may well be of significance. Unfortunately the intensity of fibrinolytic activity encountered in response to such injection in the normal subject is of a very low order.

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Chapter 5

INDUCED PLASMA FIBRINOLYTIC ACTIVITY WITHIN VEINS IN MAN

3. 5-HYDROXYTRYPTAMINE

Stevens and Lee (19) in 1884 first observed that during the clotting of shed blood a vasoconstrictor substance appeared in the serum, reaching its maximum concentration 15 minutes after clotting. The existence of this substance had been inferred by Ludwig and Schmidt (12) and Janeway, Richardson and Park in 1918 (10) considered that it was liberated from disintegrating platelets. The work of Rapport and his colleagues (15 to 18) has resulted in the isolation from serum, as a creatinine sulphate complex, of a crystalline substance, serotonin, having the chemical structure of 5-hydroxytryptamine. Their observations establish that this substance is present in sufficient quantities to account for all the vasoconstrictor effect of serum in the test which they used. It has been shown (6, 9, 14) that the only significant quantities of serotonin in the peripheral blood are to be found in the platelets.

The release of serotonin from disintegrating platelets during clotting suggested that it might have a role to play in haemostasis. The long acting vasconstrictor effect consequent upon vascular injury has been considered attributable to the release of serotonin from disintegrating platelets which agglutinate at the site of injury (2, 3, 20). Correll, Lyth, Long and Vanderpoel (5) found that the parenteral administration of serotonin retarded bleeding from incised wounds in a variety of experimental animals. They suggested that serotonin was involved in haemostasis as a humoral vasoconstrictor. Similar experiments by Correale (4) and Lecomte, Bounameaux, Fisher and Osterrieth (11) led them to propose a similar role for platelet serotonin. Fenichel and Seegers (7) concluded that in bovine plasma serotonin is the most important factor in clot retraction. On the other hand Magalini and Stefanini (13) showed that serotonin failed to enhance the clot retraction of platelet-poor plasma of man. Bigelow (1) considered that defective release of serotonin may produce purpura.

Haverback, Dutcher, Shore, Tomich, Terry and Brodie (8) found in man that reserpine given intramuscularly virtually depleted the platelets of serotonin. They found that bleeding time, blood coagulation mechanisms and capillary fragility were not altered and concluded that the serotonin in the platelets of man had no obvious role in haemostasis.

The present approach to the investigation of the function of serotonin in platelets has been from a very different point of view. The interest lay in determining if serotonin stimulated the development of fibrinolytic activity in the plasma of blood within veins.

EXPERIMENTS

Experiment 19

Observations were made on 15 subjects. A specimen of venous blood was taken as control. A sphygmomanometer cuff was then applied above the elbow and inflated to the diastolic pressure. In 5 subjects 5 µg. of 5-HT in 0.1 ml. of saline were injected into an antecubital vein. In the remaining 10 subjects the injection was made alongside an antecubital vein in doses of 5 µg. (three); 1 µg. (four); 0.05 µg. (two); and 0.01 µg. (one), each in 0.1 ml. of saline. After 5 minutes specimens of venous blood were obtained from the segment of antecubital vein employed, from a vein at the wrist of the experimental arm and from an antecubital vein of the opposite arm.

In 5 subjects (Nos. 2, 10, 11, 13 and 14) following completion of the experiment further venous specimens were obtained from the antecubital vein 15, 60 and 120 minutes after the injection of 5-HT.

Fibrinolytic activity was induced in the plasma of blood from the antecubital and the wrist veins of the experimental arm and, in all save one subject, No.6, fibrinolytic activity was increased in the specimen removed from the opposite limb (Table XVIII). The effect was found still to be present in the 5 subjects investigated, one hour after the injection (Table XIX) and, in the 2 subjects investigated, increased activity was still present 2 hours after the paravenous injection.

Table XVIII

The influence of 5-HT injected intravenously or paravenously on plasma fibrinolytic activity within veins (Experiment 19)

Subject No.	5-HT		Plasma fibrinolytic activity (Percentage of fibrin lysed per hour			
				Experimental arm		Opposite arm
	Route	Dose (µg.)	Control	Antecubital vein	Wrist vein	Antecubital vein
1	Intravenous	5.0	0.3	16.6	16.6	11.1
2	"	5.0	0.5	10.0	10.0	9.1
3	"	5.0	0.3	25.0	20.0	7.1
4	"	5.0	0.5	25.0	16.6	7.6
5	"	5.0	0.2	11.1	9.0	5.2
6	Paravenous	5.0	0.1	14.3	4.0	0.1
7	"	5.0	0.4	7.7	9.1	1.8
8	"	5.0	0.6	25.0	9.1	7.1
9	"	1.0	0.4	33.0	33.0	6.6
10	"	1.0	0.6	40.0	28.6	11.8
11	"	1.0	0.2	22.2	11.8	6.6
12	"	1.0	0.4	33.0	16.6	7.1
13	"	0.05	0.2	11.1	14.3	4.0
14	"	0.05	1.1	14.3	10.0	10.0
15	"	0.01	0.8	9.1	6.2	6.2

Table XIX

The duration of effect of 5-HT on plasma fibrinolytic activity within veins

Subject No. (Table XVIII)	5-HT		Plasma fibrinolytic activity (Percentage of fibrin lysed per hour)			
	Route	Amount (µg.) in 0.1 ml. saline	Time (minutes) after injection of 5-HT			
			5	15	60	120
2	Intravenous	5.0	10.0	-	9.1	-
10	Paravenous	1.0	40.0	28.6	14.3	-
11	"	1.0	22.2	11.1	8.3	-
13	"	0.05	11.1	-	16.6	12.5
14	"	0.05	14.3	-	12.5	9.1

Experiment 20

Experiment 11, Chapter 4 was repeated on 4 subjects substituting for adrenaline 1.0 µg. 5-HT.

The results are set out in Table XX. It will be seen on comparing these results with those obtained with ischaemia alone (Table VIII, Chapter 3), that no significant difference emerges.

Table XX

Plasma fibrinolytic activity in experimental and control arms in Experiment 20

Subject No.	Plasma fibrinolytic activity (Percentage of fibrin lysed per hour)		
	Experimental arm		Control arm
	Spec.1	Spec.2	
1	9.0	8.3	1.7
2	12.5	6.2	4.5
3	10.6	7.1	3.2
4	11.1	6.6	1.3

Comment

Erspamer (6) reported the concentration of 5-HT in human serum to range from 0.03 to 0.2 µg. with a mean of about 0.1 µg. per ml. When it is remembered that 5-HT creatinine sulphate contains approximately 40% 5-HT the lowest dose employed in the present experiments was well within the concentration encountered in serum.

The observations establish that, under the conditions of the experiment, the intravenous or paravenous injection of 5-HT results in the development of fibrinolytic activity not only within that vein but also within veins well removed proximally in the line of flow in the same arm and, for the reasons advanced in Chapter 4, possibly within veins in the opposite arm. Not only is 5-HT an exceedingly potent stimulus to the production of fibrinolytic activity within veins but its effect persists for at least 2 hours after its injection.

When injected alongside a vein in part of a limb rendered ischaemic, 5-HT failed to stimulate, as did adrenaline and acetylcholine, fibrinolytic activity greater than that which resulted from ischaemia alone.

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Chapter 6

THE EFFECT OF ATROPINE UPON THE INDUCTION OF PLASMA FIBRINOLYTIC ACTIVITY WITHIN VEINS IN MAN

In view of the demonstration that acetylcholine stimulated the production of fibrinolytic activity within veins it was manifestly desirable to determine the effect of atropine upon those stimuli known to induce such activity.

EXPERIMENTS

Experiment 21A

This was undertaken to determine the influence of atropinisation of a vein on its response to the injection of adrenaline or acetylcholine.

Observations were made on 14 subjects. A sphygmomanometer cuff was applied above the elbow of the experimental arm and inflated to the diastolic pressure. Atropine sulphate 100/ug. in 0.1 ml. saline was injected into an antecubital vein and the needle was left in the vein to the end of the experiment. After 3 minutes, to reduce venous pressure, the cuff was deflated and then reinflated to the previous pressure when in 2 subjects, 10/ug. of acetylcholine, in 4 subjects 10/ug. of adrenaline, in 3 subjects 5.0/ug. 5-HT and in the remaining 5 subjects 0.25/ug. histamine acid phosphate all in 0.1 ml. of saline, were injected through the needle. Two minutes later a specimen of blood was removed through the needle (Spec.1, Table XXI) and specimens were obtained from a vein at the wrist (Spec.2, Table XXI) and from the control arm.

In all subjects in the first 3 groups the fibrinolytic activity of Specimen 1, from the atropinised segment of vein, was significantly less than that of Specimen 2 or that of the control. In the 4th group in which histamine was employed the results were not uniform. In one case the fibrinolytic activity of Specimen 1 was greater than Specimen 2 and in 2 cases the activities of the two specimens were equal. In each of the remaining 2 cases the activity of Specimen 1 was less than that of Specimen 2. In all 5 subjects the segment of vein employed became constricted and specimens of blood were obtained only with difficulty.

Table XXI

Plasma fibrinolytic activity in experimental and control arms in Experiment 21A, Spec.1 is from the atropinised segment of vein.

Subject No.	Stimulus	Plasma fibrinolytic activity (Percentage of fibrin lysed per hour)		
		Experimental arm		Control arm
		Spec.1	Spec.2	
1	Acetylcholine (10µg.)	0.1	16.6	12.5
2	"	6.0	11.1	8.3
3	Adrenaline (10µg.)	1.0	6.0	5.0
4	"	0.6	2.0	2.0
5	"	2.0	4.0	3.0
6	"	1.2	7.6	4.1
7	5-HT (5µg.)	2.2	16.6	6.2
8	"	1.1	16.6	7.1
9	"	2.4	8.4	4.5
10	Histamine (0.25µg.)	14.0	16.6	14.0
11	"	50.0	16.6	11.0
12	"	12.5	14.3	12.5
13	"	3.2	3.2	1.4
14	"	6.6	6.6	4.1

these control values are very high

Experiment 21B

This was undertaken to determine the effect of atropine on the development of fibrinolytic activity in response to ischaemia.

Observations were made on 3 subjects. A sphygmomanometer cuff was applied above the elbow and inflated to the diastolic pressure. Atropine sulphate 100 µg. in 0.1 ml. saline was injected into the median cephalic vein and the needle left in position. After 3 minutes the cuff was inflated to 50 mm. Hg. above the systolic pressure. Ten minutes later a specimen of blood was removed through the needle (Spec.1, Table XXII) and a second specimen from the median basilic vein (Spec.2, Table XXII).

Table XXII

Plasma fibrinolytic activity in experimental and control arms in Experiment 21B. Spec.1 is from the atropinised segment of vein.

Subject No.	Plasma fibrinolytic activity (Percentage of fibrin lysed per hour)		
	Experimental arm		Control arm
	Spec.1	Spec.2	
1	1.7	14.3	-
2	0.6	11.1	-
3	0.9	12.5	-

In both subjects the fibrinolytic activity of Specimen 1 from the atropinised median cephalic vein was significantly less than that of Specimen 2 (Table XXII).

Experiment 22A

In view of the results of the experiments reported in Chapter 4 which showed that increased fibrinolytic activity develops in veins well removed proximally in the line of venous flow from the site of injection

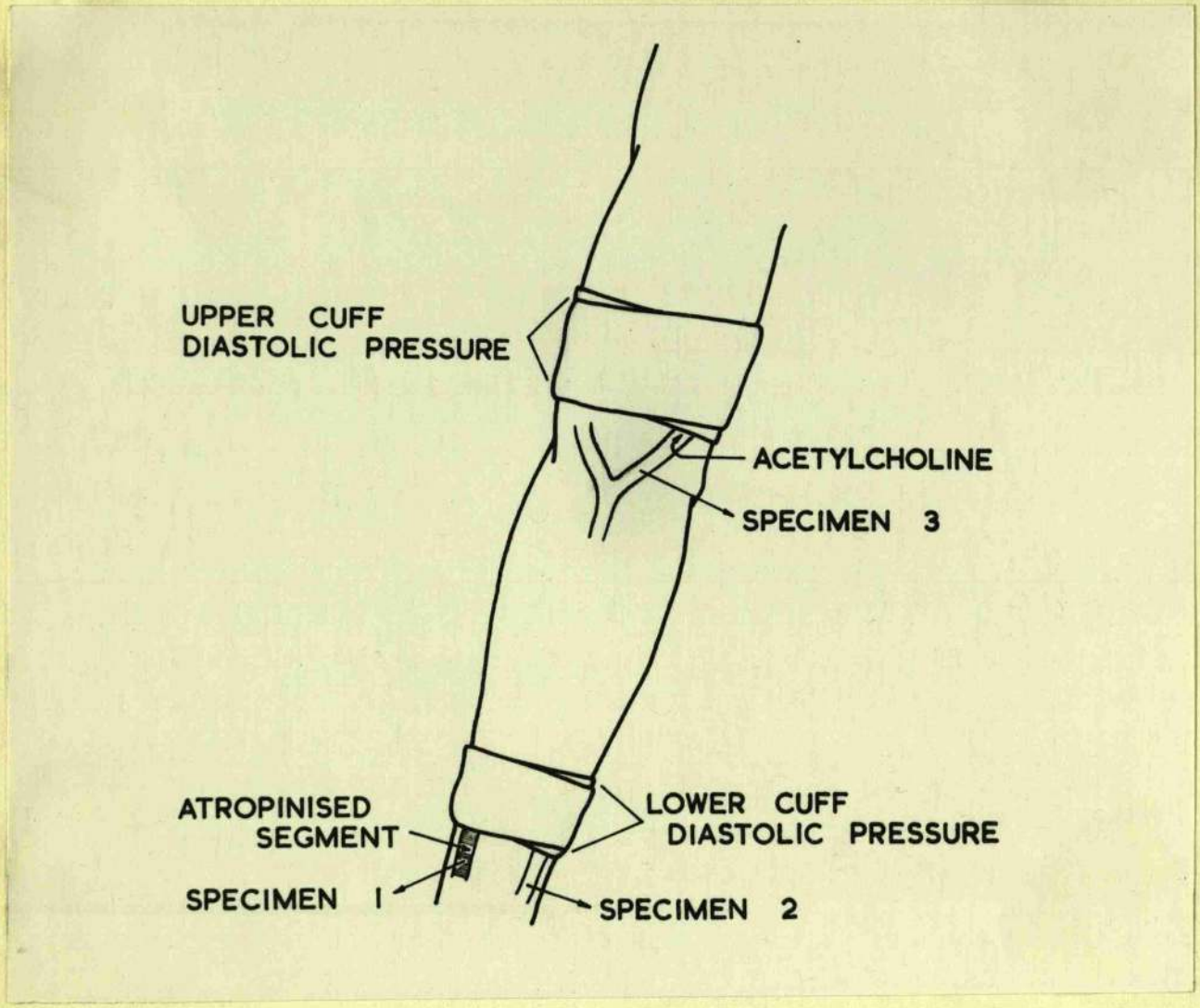

Figure 6. Experiment 22A

of either adrenaline or acetylcholine it was desirable to determine if atropine could prevent the development of this activity.

Observations were made on 2 subjects. Figure 6 illustrates this experiment. A sphygmomanometer cuff was applied to the mid-forearm of one arm and inflated to diastolic pressure. Atropine sulphate 100 µg. in 0.1 ml. saline was injected into a vein on the radial side of the wrist and the needle left in the vein. After 3 minutes the cuff was deflated to reduce venous pressure. A second cuff was applied above the elbow and

Table XXIII

Plasma fibrinolytic activity of the various specimens in Experiments 22A and 22B: Spec.1 is from the atropinised segment of vein

	Subject No.	Stimulus	Plasma fibrinolytic activity (Percentage of fibrin lysed per hour)		
			Spec.1	Spec.2	Spec.3
Experiment 22A	1	Acetylcholine (10 µg.)	4.0	16.7	14.3
	2	"	0.1	16.6	16.6
Experiment 22B	3	Ischaemia	0.0	5.0	5.0
	4	"	2.1	6.3	6.3

both cuffs were inflated to the diastolic pressure. Acetylcholine 10 µg. in 0.1 ml. saline was injected into an antecubital vein and the needle left in the vein. Two minutes later specimens of blood were obtained from the wrist vein (Spec.1, Table XXIII), from a second vein below the first cuff to the ulnar side of the wrist (Spec.2, Table XXIII) and from the antecubital vein (Spec.3, Table XXIII).

In each subject the fibrinolytic activity of Specimen 1 from the atropinised wrist vein was significantly less than that of either Specimen 2 or 3 (Table XXIII).

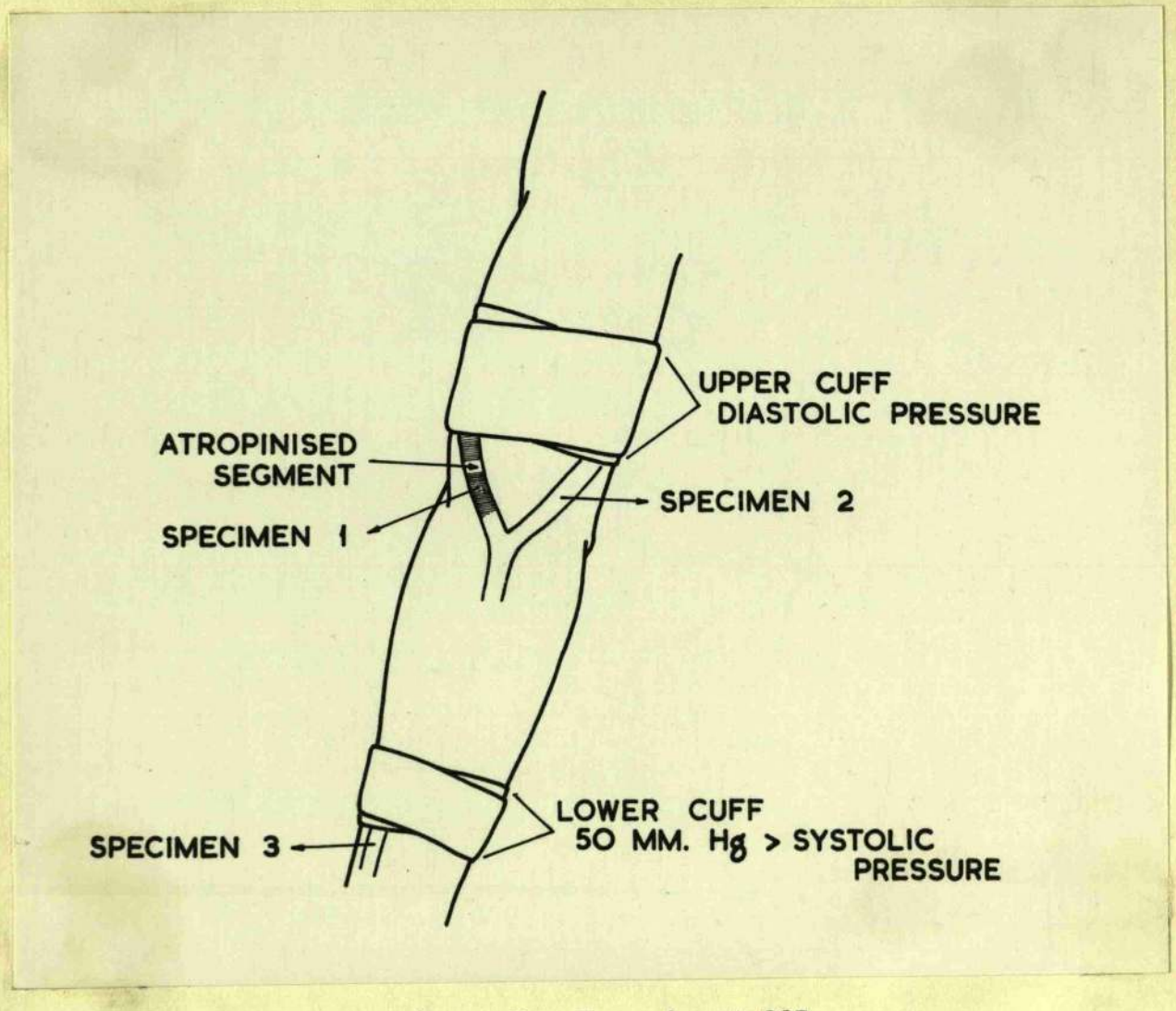

Figure 7. Experiment 22B.

Experiment 22B

In view of the results of Experiment 8, Chapter 3 in which ischaemia was employed alone it was desirable to determine whether atropine could prevent the development of fibrinolytic activity in veins distal in the line of venous flow to a cuff arresting the arterial supply.

Observations were made on 2 subjects. Figure 7 illustrates this experiment. A sphygmomanometer cuff was applied above the elbow and inflated to the diastolic pressure. Atropine sulphate 100 µg. in 0.1 ml. saline was injected into an antecubital vein and the needle left in position. After 3 minutes the cuff was deflated to reduce venous pressure and then reinflated to the diastolic pressure. At the same time a second cuff, applied to the mid-forearm, was inflated to 50 mm. Hg. above the systolic pressure. Two minutes later specimens of blood were obtained from the atropinised antecubital vein (Spec.1, Table XXIII), from a second antecubital vein (Spec.2, Table XXIII) and from a vein at the wrist (Spec.3, Table XXIII).

In each subject the fibrinolytic activity of Specimen 1 was significantly less than that of Specimen 2 or 3 (Table XXIII).

Experiment 23

For reasons commented on in Chapter 4 it appeared that the intravenous or paravenous injection of the test substances resulted in the development of increased fibrinolytic activity in the veins of the contralateral ('control') arm. The results of Experiments 22A and 22B suggested the use of atropine as a means of testing this hypothesis.

Observations were made on 4 subjects. Figure 8 illustrates this experiment. A sphygmomanometer cuff was applied to one arm (Arm 1, Table XXIV) above the elbow and 100 µg. of atropine sulphate in 0.1 ml. saline injected into the median basilic vein. The needle was left in the vein. After 3 minutes the cuff was deflated to reduce venous pressure. A second cuff was applied to the opposite arm (Arm 2, Table XXIV) and both cuffs were inflated to diastolic pressure. Acetylcholine 10 µg. in 0.1 ml.

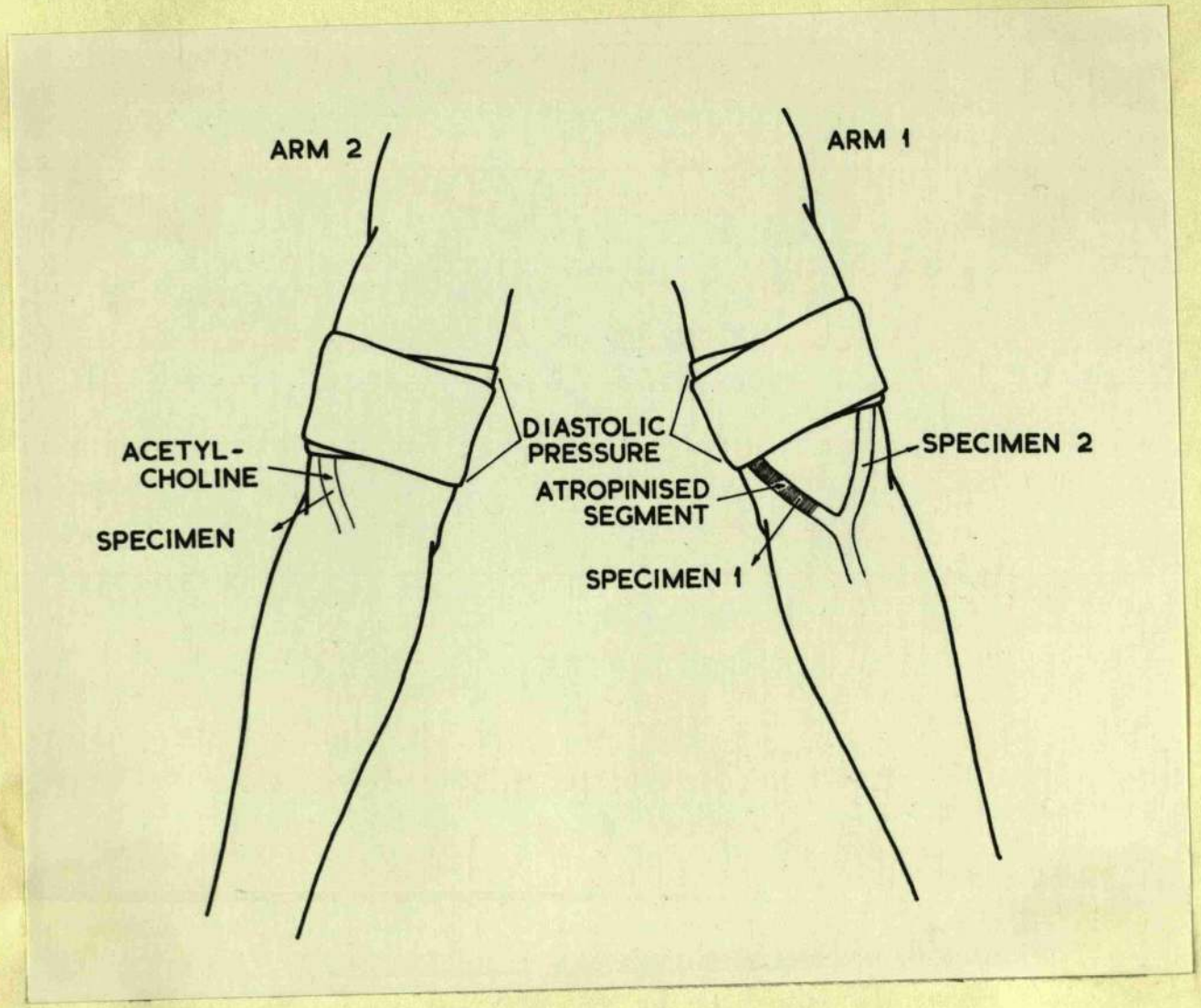

Figure 8. Experiment 23.

of saline was injected into an antecubital vein of the second arm and the needle left in position. Two minutes later a specimen of blood was obtained through each of the 2 needles. A second specimen (Spec.2, Table XXIV) was obtained from the median cephalic vein of Arm 1.

Table XXIV

Plasma fibrinolytic activity following the injection of 10 µg. of acetylcholine into an antecubital vein of Arm 2 in Experiment 23. The median basilic vein of Arm 1 was atropinised.

Subject No.	Plasma fibrinolytic activity (Percentage of fibrin lysed per hour)		
	Arm 1		Arm 2 (Antecubital)
	Spec. 1 (Median basilic vein)	Spec. 2 (Median cephalic vein)	
1	2.0	6.7	12.5
2	0.0	4.2	8.3
3	4.2	9.0	14.3
4	0.3	2.0	10.0

In each case the fibrinolytic activity of the specimen removed from the atropinised segment of vein was significantly less than that removed fro the untreated vein of the same arm.

Comment

It has been established that atropinisation of a segment of vein inhibits the production of plasma fibrinolytic activity within that vein in response to the intravenous injection of acetylcholine, adrenaline, [illegible] and 5-HT and to ischaemia. Atropine is capable of reversing the pharmacological actions of certain of the agents employed, for example, there is evidence that it can reverse the constrictor action of adrenaline (3) and the vasodilator action of acetylcholine (1, 2). This, however,

cannot be its mode of action in the case of 5-HT and it is inconceivable that it could affect ischaemia produced by arrest of the arterial supply to a limb. The evidence is the more happily considered as being consistent with the effector mechanism, responsible for the production of the fibrinolytic activity, being cholinergic. It follows that adrenaline, 5HT and also ischaemia stimulate the effector mechanism indirectly. The effect of atropine upon the response to histamine has not been established. Venoconstriction not only made it difficult to obtain specimens of blood but there was no assurance that the specimens came from the atropinised segments.

It has previously been shown that the increased fibrinolytic activity in response to the injection of test substance was not restricted to the segment of vein into which the injection was given for it was also encountered in the blood obtained from veins well removed proximally in the line of venous flow from the site of injection. This response did not occur in an atropinised segment of vein. It has also been shown previously that arrest of the arterial circulation to the distal part of an arm by an inflated cuff encircling the limb results in the development of fibrinolytic activity in the plasma of blood from veins in the proximal part of the limb not subjected to circulatory arrest. This response did not occur in an atropinised segment of vein. The evidence is consistent with the reflex excitation of a cholinergic effector mechanism which results in the production of increased fibrinolytic activity.

As commented on in Chapter 4 there was evidence which suggested the possibility that fibrinolytic activity was being stimulated in the veins of the control arm by the intravenous or paravenous injection into the experimental limb. The demonstration that fibrinolytic activity was significantly lower in the plasma of blood obtained from an atropinised segment of vein in the control arm than in that from an untreated segment, Experiment 23, affords evidence that there is a reflex excitation of a cholinergic effector mechanism in the veins of the contralateral limbs.

It is concluded that it is probable that the fibrinolytic activity which develops within veins results from the stimulation of a cholinergic effector

mechanism. Such stimulation reflexly produces fibrinolytic activity within the venous tree of the same limb and, commonly, fibrinolytic activity in the venous tree of the contralateral limb. While atropine prevents the excitation of the effector mechanism locally it does not influence the reflex response; presumably it does not act on the affectors. It follows that acetylcholine, which has been shown to excite reflexly effectors in other veins, must be possessed of an action additional to that as the effector substance.

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Chapter 7

INDUCED PLASMA FIBRINOLYTIC ACTIVITY WITHIN VEINS IN MAN

4. HEAT AND COLD, INFLAMMATION

Two chance observations, one coinciding with idle speculation, led to the experiments described in this chapter. Firstly, a subject with a furuncle on the upper arm was found to show increased fibrinolytic activity of the plasma of blood taken from a vein at the wrist. Speculation had led to consideration of the possibility that plasma fibrinolytic activity was a mechanism whereby vessels were maintained patent and free from fibrin in, for example, inflammation. The second chance observation was that the paravenous injection of a control solution which had not been warmed to body temperature induced fibrinolytic activity.

EXPERIMENTS

HEAT AND COLD

Experiment 24

Observations were made on 8 subjects. A sphygmomanometer cuff was applied above the elbow and inflated to the diastolic pressure. The hand

Table XXV

Plasma fibrinolytic activity in experimental and control arms following immersion of forearm in water at 15°C in Experiment 24

Subject No.	Plasma fibrinolytic activity (Percentage of fibrin lysed per hour)		
	Experimental arm		Control arm
	Venous blood	Capillary blood	
1	12.4	Not lysed in 24 hrs.	2.1
2	9.1	4.8	4.2
3	7.1	4.2	2.1
4	8.4	4.2	4.2
5	14.2	5.0	2.1
6	14.2	8.3	5.0
7	12.4	4.2	4.2
8	11.1	6.7	4.2

and forearm were immersed in a tank of water, the temperature of which was 15°C. No attempt was made to maintain the temperature nor was the water stirred. Fifteen minutes later specimens of blood were obtained from an antecubital vein and from a finger-tip of the experimental arm. A specimen was also obtained from an antecubital vein of the control arm.

The results are set out in Table XXV. In each case the fibrinolytic activity of the venous blood from the experimental arm was greater than that of the blood from the finger-tip which in turn was greater than (4 cases) or equal to (4 cases) that from the control limb.

Experiment 25

Experiment 4 was repeated on 7 subjects with the water at an initial temperature of 40°C.

The results are set out in Table XXVI. In one case all 3 specimens failed to lyse in 24 hours. In each of the remaining 6 subjects the fibrinolytic activity of the specimen of venous blood from the experimental

Table XXVI

Plasma fibrinolytic activity in experimental and control arms following immersion of forearm in water at 40°C in Experiment 25

Subject No.	Plasma fibrinolytic activity (Percentage of fibrin lysed per hour)		
	Experimental arm		Control arm
	Venous blood	Capillary blood	
1	Not lysed in 24 hrs.	Not lysed in 24 hrs.	Not lysed in 24 hrs.
2	25.0	"	"
3	40.0	"	"
4	10.0	"	"
5	33.0	10.0	"
6	5.8	4.1	"
7	20.0	8.3	7.1

arm was greater than that of the blood from the finger-tip or that from the control arm. In 3 of the 6 subjects the activity of blood from the finger-tip was greater than that from the control arm.

Experiment 26

The results of Experiments 24 and 25 were consistent with the major fibrinolytic activity developing within subcutaneous veins. To investigate

Table XXVII

Plasma fibrinolytic activity in experimental and control arms following the application of cold and heat to the skin overlying an antecubital vein in Experiment 26

Subject No.	Temperature of stimulus	Time Applied (mins.)	Plasma fibrinolytic activity (Percentage of fibrin lysed per hour)		
			Experimental arm		Control arm
			Spec.1	Spec.2	
1	15°C	5	20.0		4.1
2	"	"	12.5		9.1
3	"	"	3.8		0.9
4	"	"	22.2		0.8
5	"	"	10.0		7.6
6	"	2	7.1	5.0	4.1
7	"	"	16.6	8.3	5.0
8	40°C	5	10.0		3.1
9	"	"	14.3		7.1
10	"	"	9.1		5.8
11	"	"	14.3		7.1
12	"	"	14.3		5.8
13	"	2	8.3	6.6	5.8
14	"	"	20.0	8.3	5.0

this possibility the following experiment was carried out on 14 subjects. A conical aluminium container, 30 ml. in capacity, was used. The apex of this container when applied to the skin had a contact area of 5 mm. in diameter. A sphygmomanometer cuff was applied above the elbow and inflated to the diastolic pressure. The container was filled with water, in 7 instances at 15°C and in the remaining 7 at 40°C, and the apex applied to the skin over an antecubital vein without pressure. Five minutes later in 10 instances specimens of blood were obtained from that vein (Spec.1, Table XXVII) and from an antecubital vein in the control arm. In the remaining 4 instances (subjects No. 6, 7, 13 and 14, Table XXVII) after 2 minutes blood was obtained from the vein (Spec.1, Table XXVII), from a vein at the wrist (Spec.2, Table XXVII) and from an antecubital vein of the control arm.

In each subject the fibrinolytic activity of Specimen 1 was greater than that of the control. In the 4 subjects in which Specimen 2 was taken the fibrinolytic activity of this specimen was less than that of Specimen 1 but greater than that of the control.

TUBERCULIN

Experiment 27

This was undertaken to determine whether an inflammatory lesion involving the skin and subcutaneous tissue could provoke the development of fibrinolytic activity within veins. The inflammatory-necrotising response to tuberculin was employed and observations were made on 10 subjects known to have a positive tuberculin reaction. One tenth ml. of a solution of purified protein derivative of tuberculin (0.001 mg. per ml.) was injected intradermally on the volar surface of the forearm mid-way between wrist and elbow. Specimens of blood were obtained 24 and 48 hours later (Samples A and B respectively, Table XXVIII), from an antecubital vein (Spec.1, Table XXVIII), from a vein at the wrist (Spec.2, Table XXVIII) and from an antecubital vein in the control arm. Prior to the taking of the specimen a sphygmomanometer cuff, applied above the elbow, was inflated to the

Table XXVIII

Plasma fibrinolytic activity in experimental and control arms 24 (Sample A) and 48 hours (Sample B) after the intradermal injection of tuberculin in tuberculin-positive subjects in Experiment 27

Subject No.	Sample	Plasma fibrinolytic activity (Percentage of fibrin lysed per hour)		
		Spec.1	Spec.2	Control
1	A	8.3	4.1	4.1
	B	22.0	14.2	5.0
2	A	8.3	8.3	5.0
	B	12.5	5.2	4.1
3	A	8.3	8.3	7.6
	B	14.2	12.5	4.1
4	A	4.1	4.1	0.8
	B	3.4	3.2	1.9
5	A	6.6	6.6	6.6
	B	22.0	16.6	4.1
6	A	12.5	12.5	10.0
	B	22 0	14.2	4.5
7	A	5.5	4.1	1.8
	B	7.6	4.1	3.2
8	A	6.6	6.6	1.2
	B	7.6	5.0	1.4
9	A	5.5	5.5	3.8
	B	9.0	4.1	3.4
10	A	12.5	10.0	5.8
	B	14.2	7.6	4.1

diastolic pressure and maintained for one minute.

Sample A. All 3 specimens from subject No.5 showed equal fibrinolytic activity. In each of the remaining subjects the fibrinolytic activity of Specimen 1 was greater than that of the control and, in all save one, the

activity of Specimen 2 was also greater than that of the control. The activity of Specimen 1 was equal to (6 cases) and greater than (3 cases) that of Specimen 2.

Sample B. In each case the fibrinolytic activity of Specimen 1 was greater than that of Specimen 2 which in turn was greater than that of the control. On comparison of Sample B with Sample A the former showed significantly greater activity in a majority of instances.

Comment

It has been shown that the application of heat and cold over a subcutaneous vein and the inflammatory necrotising response to tuberculin stimulate the production of fibrinolytic activity within veins. These stimuli produce fibrinolytic activity within other veins of the same limb well removed from the site of initial stimulation and possibly also, for the reasons previously advanced, within veins of the contralateral limb. Since this pattern of response is similar to that encountered with other stimuli investigated it is assumed that atropinisation also would inhibit the responses local and remote to heat and cold and the inflammatory necrotising reaction to tuberculin. Lack of subjects precluded investigation.

It would seem appropriate at this juncture to review those stimuli thus far established as producing fibrinolytic activity within veins in man.

It has been shown that venous occlusion alone fails to induce fibrinolytic activity within veins but, if veins are allowed to fill by obstructing venous return and thereafter the arterial supply is arrested, fibrinolytic activity develops. This activity is significantly less than that which develops in veins distal in the line of venous flow to the site of arterial obstruction. In other words the reflex activity is greater than the direct response to the stimulus. It has been shown that the paravenous injection of adrenaline, acetylcholine or 5-HT in part of a limb to which the arterial supply has been arrested fails to produce, within

the segment of vein employed, fibrinolytic activity greater than that produced by ischaemia alone. The reflex response is again, in each case, of greater intensity than the direct response. It must be concluded that for the maximum response to occur maintenance of the arterial supply is essential. The evidence presented is consistent with the fibrinolytic activity being imparted to the plasma by effectors in the vein wall. It is difficult to see how arrest of the arterial supply could affect the efficiency of these effectors in the larger subcutaneous veins other than by arresting the circulation through the vasa vasorum in the vein wall. Assuming this it follows that relative ischaemia of a vein is the more efficient stimulus to the effectors not because it is more powerful but because it allows of the development of maximal response.

There is additional evidence which is consistent with the possibility that the circulation through the vasa vasorum has a significant part to play in the development of fibrinolytic activity within veins. It has been shown that the 'receptors' must be widely distributed since fibrinolytic activity may be induced by intradermal injection or by subcutaneous injection in an area free from visible veins. However, the activity so induced is significantly less than that encountered when the active agent is injected into or alongside a major vein. It follows that the 'receptors' are concentrated within or alongside the vein wall. If the effectors are cholinergic, and the evidence is in favour of this, then it follows that the other stimuli excite the effectors indirectly. Set out in Table A are those stimuli thus far established as inducing fibrinolytic activity within veins. Procaine is included in this table for completeness for it is shown in Chapter 8 to induce fibrinolytic activity within veins in man. Acetylcholine, the effector substance, is not included.

Group 1, Table A, consists of those stimuli which may be considered vasoconstrictor. Procaine has been so classed since Tripod (5) has shown that it shares to a lesser extent the sympathomimetic action of cocaine.

Table A

Stimuli known to induce fibrinolytic activity within veins in man

Group 1		Group 2
Cold (15°C)	Arrest of arterial supply	Histamine
Adrenaline		Heat (40°C)
Pituitrin		Inflammation
5-HT		
Procaine		

Group 2, Table A, consists of histamine, heat (40°C) and inflammation. The latter two stimuli can be regarded as releasing histamine consequently histamine may well be the common denominator to all three members of this group.

The common denominator to Groups 1 and 2 might well be the production of relative ischaemia for, although histamine is a vasodilator, in the 'wheal' response to histamine relative ischaemia might well be produced. Since venous occlusion was employed in all observations this ischaemia would be aggravated.

The efficiency of stimuli which excite indirectly the effectors, applied over, into or alongside a subcutaneous vein may well be interpreted as being due to their effect upon the circulation through the vasa vasorum of that vein which results in ischaemia of the vein wall.

The vasa vasorum of veins are highly developed. Even very small veins, with a calibre of 1 mm., have vasa vasorum. The media of a vein is better supplied than that of an artery and the nutrient vessels reach as far as the intima (1).

Little is known of the physiology of the vasa vasorum. Smith (3) reported a study by an angioplethysmokymographic technique of the variations

in flow through the vasa vasorum of isolated surviving arteries. Adrenaline, posterior pituitary extract and 5-HT constricted the vasa vasorum of swine carotid arteries. Acetylcholine, posterior pituitary extract and 5-HT constricted the vasa vasorum of both swine and human coronary arteries. Histamine although causing constriction of the arterial wall exerted no effect. Smith (2) employing the same technique showed that cold reduced greatly the flow through the vasa vasorum of a human artery. As Smith (2) rightly points out his results have been obtained on isolated arteries and they may not be applied to 'in vivo physiology' without considerable caution. A further difficulty arises in that results which apply to arteries do not necessarily apply to veins even in vitro. For example Smith and Coxe (4) in certain of their experiments found the pulmonary artery completely unresponsive to histamine whereas a pulmonary vein from the same lung was exquisitely sensitive.

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Chapter 8

THE EFFECT OF EXERCISE OF ISCHAEMIC MUSCLES ON THE INDUCTION BY ISCHAEMIA OF PLASMA FIBRINOLYTIC ACTIVITY WITHIN VEINS IN MAN

In the course of carrying out the experiments upon the production of plasma fibrinolytic activity by ischaemia, Chapter 3, an experiment was designed to determine whether the accumulation of metabolites from muscles subjected to arrest of their arterial supply played a part in the development of fibrinolytic activity of the plasma. During these experiments it was found that, if muscles in the part of the limb rendered ischaemic were exercised, fibrinolytic activity did not develop in the plasma of blood subsequently flowing through that part. The following experiments were undertaken to investigate this phenomenon.

EXPERIMENTS

Experiment 28

This was designed to determine whether the accumulation of metabolites from muscles subjected to arrest of their arterial supply played a part in the development of the fibrinolytic activity of the plasma. Experiment 1, Chapter 3, was repeated on 10 subjects but during the phase of arrest of the arterial supply the fingers of both hands were fully flexed and extended every 30 seconds. Specimens of blood were obtained as in Experiment 1 and also from a finger-tip.

The results are set out in Table XXIX. It will be seen that in each case there was no difference between the fibrinolytic activity of the plasma from the experimental arm and that from the control arm, results significantly different from those obtained in Experiment 1.

Table XXIX

Plasma fibrinolytic activity in experimental and control arms following exercise of ischaemic muscles in Experiment 28

Subject No.	Plasma fibrinolytic activity (Percentage of fibrin lysed per hour)		
	Experimental arm		Control arm (Antecubital vein)
	Spec.1 (Antecubital vein)	Spec.2 (Finger-tip)	
1	0.7	Not lysed in 24 hrs.	0.5
2	0.5	" "	0.4
3	1.1	" "	0.5
4	0.4	" "	0.6
5	0.6	" "	0.4
6	0.1	" "	0.3
7	0.5	" "	0.2
8	0.8	" "	0.3
9	0.3	" "	0.4
10	1.3	" "	1.4
Mean (S.D.)	0.6 (0.4)		0.5 (0.3)
	Experiment 1, Chapter 3		
Mean (S.D.)	14.2 (12.1)		0.5 (0.3)

Experiment 29

In order to determine whether exertion of ischaemic muscles would affect fibrinolytic activity already present in the plasma observations were made on 4 subjects. Two of these were patients with cirrhosis of the liver with high spontaneous fibrinolytic activity of the plasma. The remaining 2 were medical students who had had injected into a thigh muscle

0.5 mg. adrenaline hydrochloride 10 minutes before the experiment. Experiment 28 was repeated and specimens of blood were obtained from an antecubital vein of the experimental arm and of the control arm.

Table XXX

Plasma fibrinolytic activity in experimental and control arms following exercise of ischaemic muscles in subjects with high plasma fibrinolytic activity in Experiment 29

Subject No.	Origin of increased fibrinolytic activity	Plasma fibrinolytic activity (Percentage of fibrin lysed per hour)	
		Experimental arm (Antecubital vein)	Control arm (Antecubital vein)
1	Spontaneous in cirrhosis of the liver	21.3	20.0
2	"	33.3	33.3
3	Following adrenaline	25.0	25.0
4	"	25.0	25.0

The results are set out in Table XXX. There was no significant difference between the fibrinolytic activity of the specimen from the experimental arm and that from the control arm. Both specimens in each case were increased.

Experiment 30

It has been shown, Experiment 4, Chapter 3, that if the arterial supply to a limb is arrested the plasma fibrinolytic activity of the blood within veins increased during the phase of ischaemia. It seemed desirable to determine whether muscular exertion would prevent this increase. At the same time it was possible to determine whether muscular exertion in an ischaemic limb would prevent the increase of fibrinolytic activity of

venous blood in response to the paravenous injection of acetylcholine (Chapter 4).

Experiment 29 was repeated on 4 subjects but before arresting the arterial supply the sphygmomanometer cuff was inflated to the diastolic pressure until the superficial veins were distended. At the end of 10 minutes, with the cuff still inflated, specimens of blood were taken from a finger-tip (Spec.1, Table XXXI), from an antecubital vein (Spec.2, Table XXXI) and from an antecubital vein in the control arm. In 3 subjects 10 ug. of acetylcholine in 0.1 ml. saline were injected alongside an antecubital vein. The pressure in the cuff was reduced to the diastolic level and 2 minutes later a specimen of blood was taken from that vein (Spec.3, Table XXXI).

Table XXXI

Plasma fibrinolytic activity in experimental and control arms in Experiment 30

Subject No.	Plasma fibrinolytic activity (Percentage of fibrin lysed per hour)			
	Experimental arm			Control arm (Antecubital vein)
	Spec.1 (Finger-tip)	Spec.2 (Antecubital vein)	Spec.3 (Antecubital vein)	
1	8.3	3.1	29	0.3
2	8.3	2.7	25	0.7
3	14.3	8.3	-	0.6
4	25.0	2.3	36.6	0.4

In all subjects the fibrinolytic activity of Specimens 1 and 2 were greater than that of the control. In each of the 3 subjects investigated the paravenous injection of acetylcholine resulted in a marked intensification of fibrinolytic activity.

Experiment 31

There is reason to believe (Chapter 6) that certain stimuli which cause the development of fibrinolytic activity within veins in one limb, reflexly produce fibrinolytic activity in the venous tree of the contralateral limb. The finding of increased activity in the control specimens in Experiment 7, Chapter 3, was reminiscent of this finding in veins. The arterial punctures had been carried out following infiltration with 1% procaine and it was considered possible that either procaine or the puncture itself stimulated fibrinolytic activity within an artery.

Observations were made on 3 subjects. A sphygmomanometer cuff was applied above the elbow and inflated to the diastolic pressure. Procaine, 0.5 ml. of a 1% solution, was employed to infiltrate the skin and tissues down to the brachial artery. Two minutes later specimens of blood were

Table XXXII

Plasma fibrinolytic activity in experimental and control arms following the injection of 1% procaine in the vicinity of the brachial artery in Experiment 31

Subject No.	Plasma fibrinolytic activity (Percentage of fibrin lysed per hour)		
	Experimental arm		Control arm (Antecubital vein)
	Spec.1 (Brachial artery)	Spec.2 (Forearm vein)	
1	10	8.3	6.6
2	11.1	11.1	6.2
3	20	16.6	6.6

obtained from the brachial artery (Spec.1, Table XXXII), from a vein in the forearm (Spec.2, Table XXXII) and from an antecubital vein in the control arm.

The fibrinolytic activity of Specimen 1 in two cases was greater than

and in one case equal to that of Specimen 2. In each case the fibrinolytic activity of Speciment 2 was greater than that of the control.

Experiment 32

Experiment 31 established that either procaine or the needle puncture stimulated the production of fibrinolytic activity within arteries.

In order to determine whether procaine stimulated fibrinolytic activity within veins, observations were made on 4 subjects. A sphygmomanometer cuff was applied above the elbow and inflated to the diastolic pressure. Procaine, 0.5 ml. of a 1% solution, was injected alongside the median basilic vein. Two minutes later specimens of blood were obtained from the vein and from an antecubital vein in the control arm and the fibrinolytic activity of each specimen determined.

Table XXXIII

Plasma fibrinolytic activity in experimental and control arms following the paravenous injection of 1% procaine in Experiment 32

Subject No.	Plasma fibrinolytic activity (Percentage of fibrin lysed per hour)	
	Experimental arm (Median basilic vein)	Control arm (Antecubital vein)
1	8.3	4.1
2	10.0	5.5
3	16.7	5.9
4	6.7	4.2

The results are set out in Table XXXIII. In each case the fibrinolytic activity of the specimen from the experimental arm was greater than that from the control.

Experiment 33

There is evidence, Chapter 6, that the fibrinolytic activity which develops within veins results from the stimulation of cholinergic effectors in their wall. Since the results of Experiment 7, Chapter 3, and Experiments 31 and 32 establish that arteries respond as do veins it seemed reasonable to suppose that there are similar effectors in the arterial wall. As is discussed later the conclusion was reached that the inhibitory effect of exercise of ischaemic muscle probably was exerted on this mechanism. This experiment was undertaken to investigate one possible mode of action.

Table XXXIV

Plasma fibrinolytic activity in experimental and control arms following repetition of Experiment 2 on subjects given neostigmine intramuscularly in Experiment 33

Subject No.	Plasma fibrinolytic activity (Percentage of fibrin lysed per hour)		Dose of neostigmine (mg.)	Side-effects
	Experimental arm (Antecubital vein)	Control arm (Antecubital vein)		
1	11.1	4.2	1	Mild
2	11.1	4.2	"	"
3	20	5.8	"	"
4	16.7	5.8	"	Severe
5	33.3	12.5	1.5	"
6	15.8	5	"	Mild
7	14.2	10	"	Severe
8	20	5	"	Mild

Observations were made on 8 subjects. Neostigmine, 1 mg. in 4 cases and 1.5 mg. in the remaining 4, was injected into a thigh muscle and 30 minutes later Experiment 28 was repeated.

The results are set out in Table XXXIV. The fibrinolytic activity of each specimen from the experimental arm was greater than that from the control arm.

Comment

It has been shown that exercise of muscles in a limb deprived of its blood supply prevented the occurrence of increased fibrinolytic activity in blood subsequently flowing through the ischaemic limb. This was true not only of blood obtained from an antecubital vein but also of blood obtained from the finger-tip. It has been shown that this inhibitory effect cannot be attributed to the production of an antifibrinolysin since no effect was exerted upon fibrinolytic activity existing in the plasma prior to the experiments. It has also been shown that contraction of ischaemic muscle does not impair the response of subcutaneous venules and veins to stimuli known to induce fibrinolytic activity.

It has been shown, Chapter 3, that while arrest of the arterial supply to a finger alone resulted in the development of increased fibrinolytic activity in blood contained within that finger this increased activity did not persist following restoration of the circulation. If, however, ischaemia of the fingers was produced by arresting the arterial supply to the forearm the increased fibrinolytic activity in blood from the fingers persisted and was still present 5 minutes after restoration of the circulation. This persistence must be attributed to the inclusion of the greater mass of tissue in the ischaemic area and the increased fibrinolytic activity must either develop locally in small vessels or in the arterial tree in the ischaemic area. The source of this persistent fibrinolytic activity must be established for it is manifestly this source which dries up in the exercised ischaemic limb. The demonstration that the injection of procaine in the vicinity of an artery resulted in the development of fibrinolytic activity in blood flowing through the artery, a response similar to but more intense than that encountered in veins,

vitiates the results of Experiment 7, Chapter 3, and renders inadmissible the major evidence that the increased activity develops in the arterial tree. Nevertheless the weight of evidence is in favour of the site of major development of fibrinolytic activity, on restoration of arterial flow following ischaemia, being the arterial tree. If this is assumed then the fibrinolytic activity which persists in blood from the finger following restoration of the circulation to an ischaemic limb is imparted to it by the arterial tree in the ischaemic area. It follows that exercised ischaemic muscles exert their inhibitory effect upon arteries so located. It has been shown that the fibrinolytic activity which develops within veins in response to ischaemia results from the stimulation of possibly a cholinergic effector mechanism in their walls. The experiments with procaine renders it probable that a similar mechanism exists in the walls of human arteries. It would appear reasonable to suggest that the inhibitory effect of exercised ischaemic muscle is exerted upon this mechanism and the results of Experiment 33 support this suggestion. These results establish that the inhibitory effect of exercise of ischaemic muscles was lost presumably by the prior inhibition by neostigmine of cholinesterase activity. It is not known if this cholinesterase dominance develops within the artery itself; if so then the arterial tree within an ischaemic limb actively responds to the exercise of muscles in that limb during the phase of circulatory arrest.

Chapter 9

FIBRINOLYTIC ACTIVITY IN THE VEINS OF RABBITS' EARS

Grossi, Cliffton and Cannamela (8) found that a thrombus, produced in the marginal vein of the rabbit ear by the injection of thrombin, lysed. This appeared to afford a suitable method for an experimental study of fibrinolytic activity.

EXPERIMENTS

CONTROL OBSERVATIONS

The thrombus produced in each of 20 rabbits lysed in from 8 to 39 hours (mean = 20; S.D. = 7).

EFFECT OF 5-HT

Experiment 1

To determine whether the addition of 5-HT to the thrombus would increase the rate of lysis of that thrombus, observations were made on 28 rabbits. To the solution of thrombin the following amounts of 5-HT were added: 10 µg. (Group 1, 4 animals), 3 µg. (Group 2, 16 animals), 0.3 µg. (Group 3, 4 animals) and 0.03 µg. (Group 4, 4 animals).

The clots lysed in Group 1 in from 3 to 4 hours (mean = 3.5; S.D. = 0.5), in Group 2 in from 0.25 to 6 hours (mean = 2.9; S.D. = 1.7), in Group 3 in from 2 to 6 hours (mean = 4.5; S.D. = 1.7) and in Group 4 in from 7 to 30 hours (mean = 23; S.D. = 9).

The rate of lysis in Groups 1, 2 and 3 are significantly more rapid than in the controls ($P = < 0.001$) but on comparing one with another no significant difference emerges. The rate of lysis in Group 4 does not differ significantly from that in the controls.

Experiment 2

This was undertaken to determine whether the rate of lysis of a thrombus was reduced in the presence of thrombocytopaenia and if so, whether this reduction was prevented by the addition of 5-HT to the clot. Nine rabbits were rendered thrombocytopaenic 30 minutes prior to the

production of the thrombus. The platelet count in each animal was less than 30,000 per c.mm. The thrombi were produced in the usual way in 5 animals, Group 1. In each of the remaining 4, Group 2, 3 µg. of 5-HT were added to the solution of thrombin.

The clots lysed in Group 1 in from 48 to 84 hours (mean = 63; S.D. = 15.6) and in Group 2 in from 0.25 to 4 hours (mean = 2.4; S.D. = 1.4). The rate of lysis in Group 1 is significantly less rapid than in the controls ($P = < 0.001$) whereas that in Group 2 does not differ significantly from that encountered in Group 2, Experiment 1.

Experiment 3

Haverback, Dutcher, Shore, Tomich, Terry and Brodie (10) have shown in man that the administration of reserpine, 0.5 mg. intramuscularly twice daily, results in the removal of 5-HT from platelets by the 10th day. They have also shown in guinea-pigs that, following the administration of reserpine, 15 µg. per kg. of body weight intraperitoneally, the platelet content of 5-HT declines by 80 to 90 per cent. To determine the effect of the administration of reserpine, and presumably the effect of reducing significantly the platelet content of 5-HT, upon the rate of lysis of thrombi, 7 rabbits were given 0.1 mg. of reserpine intramuscularly twice daily for 10 days. The platelet count in each animal remained unaffected. Thrombi were then produced.

In two animals the clots lysed in 14 hours and in a third animal in 18 hours. In the remaining 4 the clots failed to lysed in 72 hours when the experiment was terminated. In each of these 4 animals a second clot was then produced in the opposite ear and to the thrombin solution were added 3 µg. of 5-HT. The rates of lysis of these second clots were not significantly different from that reported in Group 2, Experiment 1.

Experiment 4

Gaddum and Hameed (7) concluded that there were specific receptors for 5-HT in the blood vessels of the rabbit's ear which are readily

paralysed by lysergic acid diethylamide (LSD 25). To determine the influence of LSD 25, observations were made on 8 rabbits which were divided into two equal groups, Groups 1 and 2. Into the selected segment of vein isolated between clamps in each animal was injected 0.5 µg. of LSD 25 in 0.05 ml. of saline. Five minutes later the clamps were released, then reapplied. In Group 1 the clots were produced in the usual way but in Group 2 the thrombin solution contained 3 µg. of 5-HT.

The clots in both groups failed to lyse in 72 hours when the experiment was terminated.

Experiment 5

This was undertaken to determine whether 5-HT must be present in the clot itself to produce accelerated lysis. Observations were made on 6 rabbits. Clamps were applied in the usual way and into the segment of vein 3 µg. of 5-HT in 0.05 ml. saline were injected. Five minutes later the clamps were released then reapplied and the thrombus produced.

The clots lysed in from 1.5 to 6 hours (mean = 3.6; S.D. = 1.5). This result is not significantly different from that encountered in Group 2, Experiment 1.

It should be noted that the free intravenous injection of 3 µg. of 5-HT exerts no effect upon the rate of lysis of a thrombus produced in another vein.

Experiment 6

To determine the effect of the paravenous injection of 5-HT on the rate of clot lysis observations were made on 9 rabbits. They were divided into three groups of three animals, Groups 1, 2 and 3; and the quantity of 5-HT employed in each group was 0.3, 0.1 and 0.03 µg. respectively. Immediately before the production of the thrombus the 5-HT in 0.05 ml. of saline was injected parallel to and approximately 5 mm. away from the selected segment. A second clot was also produced in a vein on the other margin of the same ear.

In Groups 1 and 2 the clots formed and lysed before release of the clamps and the second clots lysed in all animals in less than 1 hour. In Group 3 the clots lysed in from 1 to 2 hours and the second clots lysed within the same time.

Experiment 7

It has been found in man that the stimulation of fibrinolytic activity in a segment of a vein in an arm produces fibrinolytic activity reflexly within the rest of the venous tree in the same arm. This experiment was carried out to determine whether such reflex activity occurred in the rabbit's ear. In each of 6 rabbits a thrombus was produced in the usual way, but immediately prior to its production 3 µg. of 5-HT in 0.05 ml. saline were injected into a segment of a vein isolated between clamps on the other margin of the ear and below the level of that selected for the thrombus.

The clots lysed in from 1 to 6 hours (mean = 2.8; S.D. = 1.7) which is significantly more rapid than the rate of lysis encountered in the controls ($P = <0.001$).

Experiment 8

It has been found in man that stimulation of fibrinolytic activity within a vein of one arm results in the reflex production of fibrinolytic activity within the veins of the opposite arm. To determine whether this phenomenon occurred in rabbits' ears, thrombi were produced simultaneously in both ears of 6 rabbits. In each animal 3 µg. of 5-HT were added to the thrombin solution producing the clot in one ear.

The results are set out in Table XXXV. In 2 animals the thrombi produced by thrombin alone lysed in 4 hours which is well below the range encountered in the control observations. In the remaining 4 animals the lysis time was within the range encountered in the control observations. The occurrence of rapid lysis in 2 animals was sufficiently suggestive of

Table XXXV

Influence of 3 µg. of 5-HT within a thrombus in one ear upon the lysis time of a thrombus produced in the opposite ear in Experiment 8

Rabbit ear	Lysis time (hours)	
	Left ear (Thrombin + 5-HT (3 µg.))	Right ear (Thrombin alone)
1	3½	4
2	3¼	28
3	2½	4
4	6	24
5	5	30
6	1½	12

contralateral reflex stimulation to prevent the use of the contralateral ear as a control in certain of the experiments.

EFFECT OF ADRENALINE AND NORADRENALINE

Experiment 9A

Since it has been shown, Chapter 4, that adrenaline stimulates the production of fibrinolytic activity within a vein, observations were undertaken to determine whether the introduction of adrenaline would augment the rate of lysis of a thrombus within a vein. Observations were made on 4 rabbits, and in each case 2 µg. of adrenaline hydrochloride were added to the solution of thrombin.

The clots lysed in 5 to 9 hours (mean = 6.3; S.D. = 2). This is a significantly more rapid lysis than that encountered in the controls ($P = < 0.01$) but it is significantly less rapid than that encountered when 5-HT in amounts of 0.3 µg. or greater is added to the thrombin solution ($P = < 0.01$).

Experiment 9B

Experiment 9A was repeated on 2 rabbits substituting for adrenaline 2 µg. of noradrenaline.

The clot lysed in 6 hours in each case.

Experiment 10A

In order to determine the effect of the paravenous injection of adrenaline upon the rate of clot lysis, Experiment 6 was repeated on 4 rabbits substituting 2 µg. of adrenaline in 0.05 ml. of saline for the 5-HT.

The clots lysed in from 4 to 9 hours (mean = 6,5; S.D. = 2). This is not significantly different from the results of Experiment 9A. Unlike 5-HT, adrenaline accelerates the rate of clot lysis by the same amount whether paravenously or intravenously injected.

Experiment 10B

Experiment 10A was repeated on 4 rabbits substituting for adrenaline 2 µg. of noradrenaline. In 2 cases the clots lysed in one hour and, in the remaining 2 in 2 hours.

Experiment 11A

This was undertaken to determine whether adrenaline accelerated the rate of clot lysis in virtue of its vasconstrictor effect. Burn and Dutta (2) have shown that this effect can be converted to a dilator effect by priscol.

Observations were made on 4 rabbits. Priscol, 100 µg. in 0.05 ml. of saline, was introduced into the selected segment of vein between clamps. After 5 minutes both clamps were released, reapplied and a thrombus was produced. The solution of thrombin contained 2 µg. of adrenaline.

The clots lysed in 3 rabbits in 1 hour and in the fourth in 2 hours, which is well below the range encountered not only in the controls but also in Experiment 9A.

Experiment 11B

The results of Experiment 11A are consistent with the augmentation by priscol of the rate of clot lysis. Priscol has, in addition to its adrenergic blocking effect, parasympathomimetic and histamine-like actions (15).

To determine the effects of priscol on the rate of clot lysis Experiment 11A was repeated on 3 rabbits but no adrenaline was added to the solution of thrombin.

The clots lysed in 2 animals in 3 hours and in the third in 4 hours which is significantly more rapid than in the controls ($P =< 0.001$).

EFFECT OF ACETYLCHOLINE

Experiment 12

To determine whether the addition of acetylcholine to the clot would increase the rate of clot lysis, observations were made on 5 rabbits. Acetylcholine, 100 µg., was added to the solution of thrombin. To determine whether acetylcholine stimulated the production of fibrinolytic activity in other veins of the same ear, a second clot was produced in a vein on the other margin of the same ear.

The clots lysed in from 3 to 4 hours (mean = 3.5; S.D. = 0.5) which is significantly more rapid than in the controls ($P =< 0.001$) but is not significantly different from that encountered in Group 2, Experiment 1. The second clots lysed in from 8 to 18 hours (mean = 10; S.D. = 3.8). This is significantly more rapid that in the controls ($P =< 0.01$).

Experiment 13

To determine the effect of acetylcholine injected paravenously on the rate of lysis of a clot Experiment 6 was repeated on 4 rabbits substituting 10 µg. of acetylcholine in 0.05 ml. of saline for the 5-HT.

The clots formed and lysed before release of the clamps. The clot in the other vein in each case lysed within 30 minutes. It would appear that the paravenous injection of acetylcholine is a more powerful stimulus

than intravenous injection to the production of fibrinolytic activity not only locally but also in other veins of the same ear.

Experiment 14

The action of acetylcholine in stimulating fibrinolytic activity reflexly is consistent with its having an action additional to that as the effector substance for it has been shown, both in the rabbit and in man, that the local stimulation of effectors is not essential to the reflex stimulation of effectors in other veins. It is known that acetylcholine, under certain circumstances, has a vasoconstrictor action (2, 12) and it has been shown that this action in the vessels of the rabbit ear is due to the release of noradrenaline from these vessels (3). Burn and Rand (3) found that if large doses of reserpine are administered to rabbits a noradrenaline-like substance, present in the skin of the ears of untreated rabbits, disappeared.

Observations were made on 4 rabbits. They received 1.5 mg./kg. of reserpine by intraperitoneal injection on the first day and 5 mg./kg. by intravenous injection on the second. Four hours after the second injection Experiment 13 was repeated on all 4 animals.

In each animal the first clots formed and lysed before release of the clamps. The second clots lysed within 45 minutes.

EFFECT OF HISTAMINE

Experiment 15

To determine whether the addition of histamine to the clot would increase the rate of clot lysis observations were made on 5 rabbits. Histamine, 0.25 µg., was added to the solution of thrombin.

The clots lysed in from 2 to 4 hours (mean = 2.4; S.D. = 0.8) which is significantly more rapid than in the controls ($P = < 0.001$).

Experiment 16

To determine the effect of histamine injected paravenously on the

rate of lysis of a clot Experiment 6 was repeated on 3 rabbits substituting 0.25 µg. of histamine in 0.05 ml. of saline for the 5-HT. One clot only was produced in each case.

The clots formed and lysed before release of the clamps.

Experiment 17

To determine whether histamine stimulated the production of fibrinolytic activity in other veins of the same ear Experiment 7 was repeated on 4 rabbits substituting 0.25 µg. of histamine in 0.05 ml. of saline for the 5-HT.

The clots lysed in from 2 to 5 hours (mean = 3; S.D. = 1.2) which is significantly more rapid than in the controls ($P = < 0.001$).

EFFECT OF 5-HT AND HISTAMINE

Experiment 18

Since platelets not only contain 5-HT but also histamine (11) it was desirable to determine whether histamine would augment the increased rate of lysis of thrombi encountered when 5-HT was added to the solution of thrombin. Observations were made on 4 rabbits. To the solution of thrombin 0.3 µg. of 5-HT and 0.25 µg. of histamine were added.

The clots formed and lysed before release of the clamps.

EFFECT OF ATROPINE

Experiment 19

This experiment was undertaken to determine whether atropinisation of a segment of vein would inhibit the abnormally rapid lysis of thrombi which resulted when 5-HT was added to the solution of thrombin. Observations were made on 4 rabbits. Atropine, 100 µg. in 0.05 ml. of saline, was introduced into the selected segment of vein between the clamps. After 5 minutes both clamps were released, reapplied and the thrombus produced. The solution of thrombin contained 3 µg. of 5-HT.

The clots lysed in 12 to 24 hours (mean = 16.7; S.D. = 5.1). This

is significantly slower than the rate encountered in the absence of atropinisation (Group 2, Experiment 1, $P = <0.001$).

Experiment 20

This was undertaken to determine whether atropinisation would inhibit the abnormally rapid lysis of thrombi encountered when 5-HT was injected into another vein of the same ear. Experiment 7 was repeated on 4 rabbits but 5 minutes before production of the thrombi, 100 µg. of atropine in 0.05 ml of saline were injected into the selected segment. The clamps were released and reapplied before production of the thrombi.

The clots lysed in from 16 to 36 hours (mean = 25; S.D. = 7) which is significantly slower than in Experiment 7 ($P = <0.001$).

EFFECT OF DENERVATION

Experiment 21

Blood vessels of all sizes are possessed of a complex terminal nerve network in their walls. The profusion of this network is a surprising contrast to the relative insignificance of the extrinsic vascular nerves. Its presence in veins is not dependent on the presence of smooth muscle in their walls for it is found even in muscle-free veins. Franklin (6) states that "one can assume an action of the central nervous system upon every single cell in the vessel wall". Section of the post-ganglionic sympathetic fibres results in degeneration of the extrinsic vascular nerves but not in degeneration of the terminal nerve network (14, 16, 17).

The great auricular and great occipital nerves were cut at the root of one ear and the homolateral stellate and superior sympathetic ganglia were excised in 4 rabbits. Three weeks later a thrombus was produced by a thrombin solution containing 100 µg. of acetylcholine. A second clot was produced in a segment of vein on the other margin of the same ear by thrombin solution alone.

The first clots lysed in from 1 to 4 hours (mean = 2.5; S.D. = 1.1)

which is not significantly different from the rate of lysis encountered in Experiment 12. The second clots lysed in from 18 to 26 hours (mean = 22; S.D. = 28) which is significantly longer than the rate of lysis of the second clots in Experiment 12 ($P = <0.05>0.02$) but is not significantly different from the controls.

EFFECT OF TRAUMA

Experiment 22

Kwaan, McFadzean and Cook (13), in observations on patients with increased spontaneous fibrinolytic activity of the plasma, found that such activity was significantly reduced 4 hours after surgical operation and disappeared within 24 hours. In order to determine the influence of surgical operation on the lysis of a thrombus experimentally produced in a vein, observations were made on 8 rabbits. Under nembutal anaesthesia the spleen was removed in 4 animals and the right kidney in the remaining four. Twelve hours after operation a thrombus was produced in each animal.

In all animals the thrombi persisted until the 8th day when a biopsy was taken. On histological examination there was no evidence of organisation of the clot.

EFFECT OF CORTICOTROPHIN

Experiment 23

In order to determine the influence of the administration of corticotrophin on the lysis of thrombi each of 26 rabbits was given intramuscularly 3 units of corticotrophin per kg. of body weight 8-hourly for 48 hours before production of the thrombus and subsequently throughout the duration of the experiment.

In one animal the clot lysed after 48 hours. In the remaining 25 the thrombi persisted until the 8th day when a biopsy was taken. On histological examination there was no evidence of organisation of the clot.

These observations have been extended and it has been found that if

corticotrophin is given for 48 hours before, but not subsequent to the production of the thrombus, the thrombus will persist. If, however, corticotrophin is given for 24 hours before production of the thrombus, the thrombus will lyse within the time encountered in untreated animals despite continued administration of the corticotrophin.

Experiment 24

This was undertaken to determine whether the administration of corticotrophin would result in the inhibition of the abnormally rapid lysis of thrombi which occurred when 5-HT was added to the solution of thrombin. Each of 8 rabbits was given corticotrophin as in Experiment 23 but in the production of the thrombus 3 µg. of 5-HT were added to the solution of thrombin.

In one animal the clot lysed in 48 hours. In the remaining seven it persisted until the 8th day when biopsy was taken. On histological examination there was no evidence of organisation of the clot.

This experiment was repeated employing progressively larger quantities of 5-HT and it has been found that the action of 20 ug. was inhibited.

Experiment 25

This was undertaken to determine whether the administration of corticotrophin would result in the inhibition of the abnormally rapid lysis which occurred when 5-HT was injected paravenously. Experiment 6 was repeated on 6 rabbits given corticotrophin as in Experiment 23. The animals were divided into two equal groups. The following quantities of 5-HT in 0.05 ml. of saline were injected paravenously, 3 µg. in Group 1 and 0.3 µg. in Group 2.

In Group 1 the clots formed and lysed before release of the clamps. In Group 2 the clots lysed in 4, 5 and 5 hours respectively. While some inhibitory action was exerted this was markedly less than that exerted on 5-HT given intravenously.

Experiment 26

This was undertaken to determine whether the administration of corticotrophin would result in the inhibition of the abnormally rapid lysis of thrombi which resulted when adrenaline was added to the solution of thrombin. Experiment 9A was repeated on 4 rabbits given corticotrophin as in Experiment 23.

The clots lysed in from 8 to 36 hours (mean = 17.7; S.D. = 11.2). It is to be remembered that the accelerated clot lysis encountered when adrenaline is added to the thrombin solution in untreated animals (Experiment 9A) represents the sum of the action of adrenaline and that of the 5-HT released within the clot. In view of the results of Experiments 21 and 22 it may be taken that the action of the 5-HT is inhibited; consequently, it must be concluded that the lysis recorded is due to adrenaline and that the administration of corticotrophin fails to inhibit the action of adrenaline.

Experiment 27

This was undertaken to determine whether the administration of corticotrophin would result in the inhibition of the abnormally rapid lysis of thrombi which resulted when histamine was added to the solution of thrombin. Experiment 15 was repeated on 4 rabbits given corticotrophin as in Experiment 23.

The clots lysed in from 7 to 72 hours (mean = 23.8; S.D. = 27.8). For the reasons advanced in Experiment 24 above it must be concluded that corticotrophin fails to inhibit the action of histamine.

Experiment 28

The inhibitory effect of corticotrophin on the action of 5-HT introduced into a vein, the relatively minor inhibitory effect upon the action of 5-HT injected paravenously suggested the possibility that the 5-HT when introduced into a vein in corticotrophin-treated animals failed to reach the receptors in the vessel wall or in the immediately adjacent

tissue. There is a naturally occurring enzyme, mono-amine oxidase, an important function of which is the oxidation of 5-HT (Blaschko, (1)). Thompson and Tickner (18) reported its occurrence in blood vessels. Although no information was available as to whether this enzyme occurred in increased quantities following the administration of corticotrophin it seemed possible that this might be the case. To test this possibility an inhibitor of mono-amine oxidase, S.K.F. 42 (Fellows and Bernheim, (5)) was employed. Four rabbits were given corticotrophin as in Experiment 23. Into the segment of vein isolated between clamps were injected 50 µg. of S.K.F. 42 in 0.05 ml. of saline. After 5 minutes the clamps were released, reapplied and clots produced by a thrombin solution containing 3 µg. of 5-HT.

The clots formed and lysed before release of the clamps.

Experiment 29

This was undertaken to determine the influence of S.K.F. 42 upon the lysis of clots produced in untreated rabbits. Observations were made on 4 rabbits. S.K.F. 42 was injected as in Experiment 28. In two animals thrombi were produced in the usual way and in the remaining two, 0.03 µg. of 5-HT was added to the thrombin solution. In the former the clots lysed in each case in 5 hours and in the latter they lysed in each case in 1 hour.

EFFECT OF CHOLESTEROL

Experiment 30

Greig (9) reported the inhibition in man of spontaneous fibrinolytic activity of the plasma by a fatty meal. In order to determine the effect of feeding cholesterol on the lysis of thrombi in veins 21 rabbits were fed cholesterol, 1 g. per kg. of body weight in oil daily. On the 10th day a clot was produced.

The clots lysed in 6 animals within the time encountered in controls. The veins were normal on histological examination. In the remaining 15

animals the clots did not lyse, but a free flow through the clot was established in 72 to 122 hours (mean = 86; S.D. = 7). A biopsy was taken 10 days after production of the thrombus and on histological examination the veins were abnormal. The thrombus in each case had become organised and this had resulted in variable and irregular thickening of the intima. In the interstices of the granulation tissue 'foam' cells were present in varying numbers.

Experiment 31

This was undertaken to determine whether the feeding of cholesterol could inhibit the abnormally rapid lysis of thrombi which occurred when 5-HT was added to the solution of thrombin. Ten rabbits were fed cholesterol as in Experiment 30. On the 10th day a thrombus was produced. To the thrombin solution were added 3 µg. of 5-HT. The clots lysed in from 1 to 18 hours (mean = 7.6; S.D. = 5.9). This is significantly slower than the lysis time encountered in Group 2, Experiment 1 ($P = < 0.01$).

Experiment 32

In order to determine whether the feeding of cholesterol would inhibit the rapid lysis of thrombi which occurred when adrenaline was added to the solution of thrombin, Experiment 9A was repeated on 4 animals fed cholesterol for 10 days as in Experiment 30.

The clot in one animal lysed in 24 hours. In the remaining three the clots did not lyse but free flow was established through the clots in between 3 to 4 days. A biopsy, taken on the 10th day, in each of these three animals showed appearances indistinguishable from those reported in Experiment 30.

Comment

It has been shown that either the reduction in the number of platelets in the peripheral blood or reduction in the content of 5-HT in platelets, the number of platelets being unaffected, prolongs the lysis

time of a clot produced in a vein of the rabbit's ear. In both cases this effect is corrected by the addition of 5-HT to the clot. It is reasonable to conclude that platelets in virtue of their content of 5-HT are responsible for the lysis of such a clot.

It has been shown that, as in subcutaneous veins in man, 5-HT stimulates a cholinergic effector mechanism in the walls of veins in the rabbit's ear. This results in the development within these veins of fibrinolytic activity of such intensity as to result in the rapid lysis of clotted whole blood. Such stimulation reflexly produces through similar effectors fibrinolytic activity within other veins of the same ear and probably also in veins of the opposite ear. That the former is indeed a reflex stimulation is established by Experiment 19. The pathway is presumably the extrinsic vascular nerves.

Erspamer (4) reported the concentration of 5-HT in rabbit serum to be 3.53 µg. per ml. and, in the same animal, Humphrey and Jaques (11) a concentration of 7.5 µg. per 10^9 platelets. The present observations indicate that the maximum activity of the effectors, obtainable by the intravenous injection of 5-HT, occurs with a solution of 5-HT containing in 0.05 ml. perhaps less than 0.3 µg. but greater than 0.03 µg., concentrations of 6 and 0.6 µg. of the creatinine sulphate per ml., respectively. While in the present experiments it is realised that this maximum activity represents possibly the sum of two stimuli, that of the injected 5-HT and that of the 5-HT from disintegrating platelets within the clot, it seems reasonable to suggest that an amount of 5-HT well within the range of the normal content of platelets would be capable of stimulating maximum activity. In the experiments a highly abnormal form of clotting has been employed. First, there is a sudden massive conversion of fibrinogen to fibrin and the rate of lysis of such a clot does not afford a measure of that of a clot produced in the normal process of coagulation. Fibrin in the latter is formed more slowly and is the more rapidly lysed (Kwaan, McFadzean and Cook, 13). Secondly, the platelets are dispersed

throughout the clot whereas under pathological conditions platelets would agglutinate on the injured endothelium and this would be most efficient in bringing 5-HT in contact with the vessel wall. This second defect in the technique is corrected by the introduction of readily available 5-HT.

It has been shown that the addition of histamine and 5-HT to the thrombin solution results in a very much faster rate of clot lysis than when 5-HT is employed alone. This is of great importance for platelets contain histamine as well as 5-HT. Humphrey and Jaques (11) found rabbit platelets to contain 2 to 6.7 (mean = 3.5) ug. per 10^9 platelets. They concluded that, in the rabbit, platelets contribute practically all the histamine of the blood. Since it is known that corticotrophin does not inhibit the response to histamine the results of Experiment 23 establish that the histamine released from disintegrating platelets plays no apparent part in the lysis of thrombi produced experimentally by the injection of thrombin. It seems reasonable to conclude however, that when platelets agglutinate on an injured endothelium, the histamine there released would exert influence and augment the response to 5-HT.

The paravenous injection of 5-HT has been shown to produce significantly more rapid lysis than the intravenous injection of significantly larger quantities; consequently, it would appear that the receptors are more readily reached by the former route. The inhibition of mono-amine oxidase in the wall of a vein has been shown to abolish this discrepancy between intravenous and paravenous injection. This is consistent with the hypothesis that 5-HT from within the vein has to traverse the vein wall to produce maximal stimulation. In the untreated animal in so doing it is subjected to the inhibitory action of mono-amine oxidase which restricts its sphere of activity and consequently the intensity of the response. Gaddum and Hameed (7), employing the perfused vessels of the rabbit ear in a study of specific antagonists to 5-HT, concluded that there were specific 5-HT receptors in the blood vessels of

the rabbit ear which are easily paralysed by LSD 25. The results of the observations employing LSD 25 indicate that the same category of receptors is responsible for initiating the mechanism which results in the development of fibrinolytic activity within veins in response to 5-HT. It follows that these receptors are concentrated in but not restricted to the outer aspect of the vein wall. It should be noted that Gaddum and Hameed (7) concluded that potentiation of the vasoconstrictor effect of 5-HT was achieved by the inhibition of mono-amine oxidase.

It has been shown that the administration of corticotrophin results in the inhibition of the action of 5-HT given intravenously even in quantities approximately 80 times that producing the maximum rate of clot lysis by this route in untreated animals. The results of the observations employing S.K.F. 42 are consistent with this inhibition being exerted by mono-amine oxidase in the vessel wall. As a corollary, suprarenal cortical activity consequent upon the administration of corticotrophin must result either in increase in the concentration or enhancement of the action of this enzyme. It may be concluded that the persistence of thrombi in rabbits following operation is due to the same inhibitory mechanism.

Acetylcholine, adrenaline, noradrenaline, priscol and histamine have been shown to stimulate within veins the development of fibrinolytic activity of such intensity as to result in the rapid lysis of clotted whole blood. Such stimulation reflexly excites fibrinolytic activity within other veins of the same ear. The intravenous and paravenous injection of adrenaline were found to be equally effective whereas the paravenous injection of noradrenaline, acetylcholine or of histamine, like 5-HT, produced significantly more rapid lysis than the intravenous injection. The action of acetylcholine in stimulating reflex activity suggests that it might have an action additional to that as the effector substance, for it has been shown, both in the rabbit and in man, that the local stimulation of effectors is not essential to the reflex stimulation of effectors in other veins. It has been shown that if acetylcholine has an

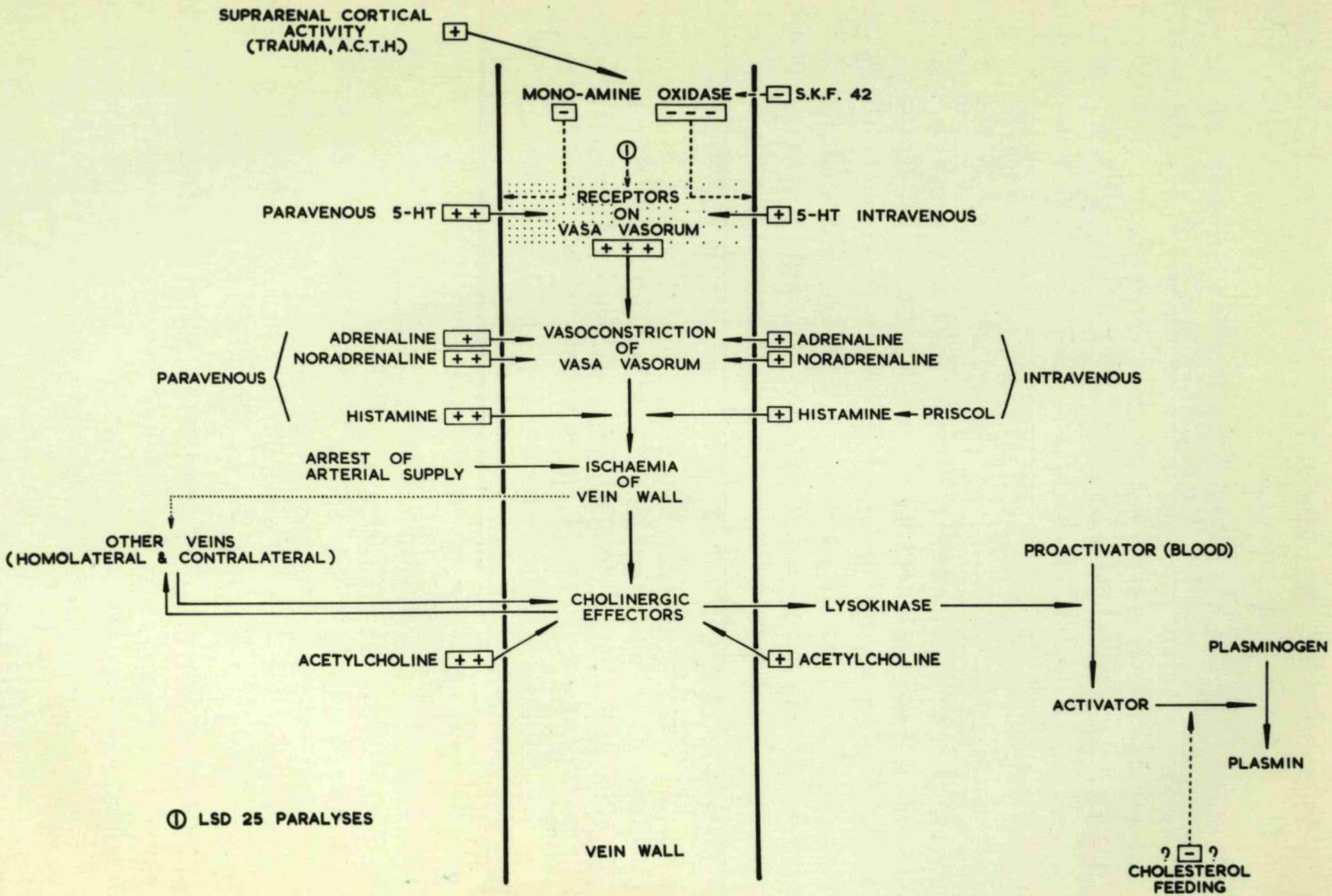
SUPRARENAL CORTICAL ACTIVITY (TRAUMA, A.C.T.H.)
MONO-AMINE OXIDASE
S.K.F. 42
RECEPTORS ON VASA VASORUM
PARAVENOUS 5-HT
5-HT INTRAVENOUS
VASOCONSTRICTION OF VASA VASORUM
PARAVENOUS
ADRENALINE
NORADRENALINE
ADRENALINE
NORADRENALINE
INTRAVENOUS
HISTAMINE
HISTAMINE
PRISCOL
ARREST OF ARTERIAL SUPPLY
ISCHAEMIA OF VEIN WALL
OTHER VEINS (HOMOLATERAL & CONTRALATERAL)
PROACTIVATOR (BLOOD)
CHOLINERGIC EFFECTORS
LYSOKINASE
PLASMINOGEN
ACETYLCHOLINE
ACETYLCHOLINE
ACTIVATOR
PLASMIN
LSD 25 PARALYSES
VEIN WALL
? ?
CHOLESTEROL FEEDING

Figure 10

additional action it is not due to its ability to release noradrenaline from vessels in the rabbit ear. In the light of the evidence available it would appear reasonable to conclude that the stimulation of effectors reflexly excites effectors in other veins.

The action of adrenaline and of histamine in accelerating the rate of clot lysis, unlike that of 5-HT, were not inhibited by corticotrophin. The inhibition resulting from the feeding of cholesterol affects both the response to 5-HT and to adrenaline. As is to be expected, the latter, the weaker stimulus, is the better inhibited. The inhibition by cholesterol also differs from that produced by corticotrophin in that it invariably permits of organisation and recanalisation of the thrombus. The nature of the inhibition by cholesterol has not been established but, thus far, the only circumstance in which the action of adrenaline and of 5-HT have been inhibited has been in the presence of the 'antifibrinolytic' activity of the plasma encountered in patients with primary carcinoma of the liver (Chapter 10).

Figure 10 is a diagrammatic presentation of an interpretation, based on the discussion in Chapter 7, of the results of the experiments described in this chapter. Arrest of the arterial supply has been included for ease in presentation although the effect of such on the rabbit has not been determined. It has been shown that 5-HT acts probably upon specific receptors readily paralysed by LSD 25. In the diagram the existence of specific receptors of 5-HT has been postulated to exist in the vasa vasorum.

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Chapter 10

THE RAISON D'ETRE OF THE FIBRINOLYTIC MECHANISM IN VEINS

It was manifestly desirable to determine whether inhibition of the mechanism which imparts fibrinolytic activity to the plasma of blood within vessels or the inhibition of the activity so produced would result in intravascular clotting. From the preceding observations the raison d'être of the mechanism can only be inferred.

EXPERIMENTS ON RABBITS

Experiment 33

This experiment was carried out to determine whether the inhibition of the response of the mechanism to 5-HT by corticotrophin would result in a fibrin thrombus forming within a vein following an injury to it.

Observations were made on 6 rabbits given corticotrophin as in Experiment 23. At the end of 48 hours a 25 gauge hypodermic needle was inserted into a marginal vein of an ear and withdrawn.

In none of the veins did a thrombus develop.

Experiment 34

This was undertaken to determine whether slowing of venous flow prior to injury to the vein would exert an influence on the response to injury.

Observations were made on 8 rabbits given corticotrophin as in Experiment 23, Chapter 9. At the end of 48 hours black braided silk (3/0) mounted on an 'atraumatic' needle was passed through the skin, around a marginal vein of the ear and tied, thereby obstructing the vein. Experiment 33 was then repeated, the needle being inserted 1 cm. proximal in the line of venous flow to the ligature.

Blood welled from the needle puncture but within 90 seconds bleeding ceased. In each animal within 15 minutes a red blood clot had formed which completely filled the lumen of the vein and extended from the ligature to a point a variable distance proximal in the line of venous flow to the

needle puncture. In 3 instances the clots lysed some time between 12 and 24 hours after their production. In the remaining 5 the clot was present, although reduced in length and in 2 instances fragmented, 24 hours after its production when the experiment was terminated.

To serve as control observations the experiment was repeated on 6 rabbits which were not given corticotrophin. In all 6 rabbits blood welled from the needle puncture and, as in the experimental group, bleeding ceased within 90 seconds. In none of the rabbits did a thrombus form

Experiment 35

Experiment 34 was repeated on 4 rabbits given reserpine as in Experiment 3, Chapter 9. In each animal a red blood clot formed as in Experiment 34. In one instance the clot lysed 20 hours later whereas in each of the remaining 3 it persisted for 24 hours when the experiment was terminated

Experiment 36

Observations were made on 4 rabbits. A segment of vein was atropinised as in Experiment 19, Chapter 9. Before release of the clamps the end of the segment of vein distal in the line of venous flow was ligated as in Experiment 34.

In each rabbit a thrombus formed which extended from the ligature to the needle puncture but not above it. The clots lysed in all animals some time between 6 and 17 hours after their production.

OBSERVATIONS ON MAN

Since the first report by Cosgriff and his colleagues (2, 3) the possibility of thrombo-embolic phenomena occurring as a complication of corticotrophin therapy has been recognised. It is also considered possible that corticotrophin therapy in patients with cirrhosis of the liver may provoke thrombosis of the portal vein (4, 8). It has been shown that such treatment, and also surgical operations, notably splenectomy, cause the

disappearance of the increased plasma fibrinolytic activity encountered in the blood of patients with cirrhosis of the liver (8, 9). It is impossible to draw conclusion since the diseases for which the treatment is given may give rise to intravascular thrombosis.

No opportunity has arisen of making a histological study of skin puncture wounds in patients receiving treatment with corticotrophin. In the course of observations on the increased spontaneous fibrinolytic activity of the plasma encountered in cirrhosis of the liver it was found that this activity disappeared following the development of hepatocarcinoma. It is well recognised that venous thrombosis, notably of subcutaneous veins, occurs in many forms of carcinoma but a search of the literature failed to reveal a report of its occurrence in hepatocarcinoma. However, in a series of 250 patients suffering from hepatocarcinoma McFadzean (13) found it to be present in 28, an incidence of 11%. In none of these patients was there demonstrable abnormality in clotting. An investigation of the fibrinolytic mechanism manifestly was indicated.

MATERIAL AND ADDITIONAL METHODS

Observations were made on patients with uncomplicated cirrhosis of the liver and on patients with cirrhosis of the liver complicated by the development of hepatocarcinoma. The diagnosis in each group was established by aspiration needle biopsy and, in the latter, confirmed at autopsy. The healthy controls were volunteer medical students and members of the staff.

Adrenaline test

Adrenaline, 0.5 ml. of a 1/1000 solution was injected subcutaneously in the thigh and the fibrinolytic activity of a specimen of blood obtained 20 minutes later from an antecubital vein determined.

'Antifibrinolytic' activity

Observations were made with plasma, serum and/albumin fraction of the plasma. The albumin fraction was prepared from plasma by the method of

Macfarlane and Pilling (12); the pH of the final solution being adjusted to 7.4. Plasma with high fibrinolytic activity was obtained from healthy subjects following the paravenous injection of adrenaline or of 5-HT. To 5 tubes were added 0.1 ml. of this active plasma and such volume of the fraction under test as to yield ratios of test fraction-fibrinolytic plasma of 1/1, 1/2, 1/5, 1/10, 1/20. A sixth tube containing 0.1 ml. of fibrinolytic plasma served as control. To each tube sufficient veronal-acetate buffered saline, pH 7.4, was added to bring the final dilution of fibrinolytic plasma to 1/25. The tubes were then clotted with thrombin and observed at hourly intervals for lysis. The results are expressed as the percentage reduction in fibrinolytic activity of the active plasma at each dilution.

Plasmin and antiplasmin activity

Chloroform-activated plasmin was prepared by the method of Rocha e Silva and Rimington (16) as modified by Bidwell (1). Antiplasmin activity was determined by adding 0.2 ml. of plasma under test to varying amounts of plasmin: saline buffered to pH 7.4 being added to bring the volume to 5.0 ml. The mixture was then clotted with thrombin and incubated at 37°C and the lysis times determined.

'Antifibrinolytic' activity of tissue

A specimen of tumour was obtained from each of 2 patients with hepatocarcinoma within an hour of death: specimens of 2 apparently normal livers similarly obtained were used as controls. Each specimen was finely minced and extracted by the addition of 10 times its weight of saline buffered to pH 7.4, centrifuged and the supernatant employed to determine 'antifibrinolytic' activity as described above.

RESULTS

Spontaneous Fibrinolytic Activity and Fibrinogen levels

The spontaneous fibrinolytic activity and the fibrinogen levels

encountered in 35 patients with hepatocarcinoma, in 60 healthy controls and in 60 control patients with cirrhosis of the liver are set out in Table XXXVI. There was no difference between the fibrinolytic activity in the patients with hepatocarcinoma and that in the healthy controls. The fibrinolytic activity in the controls with cirrhosis of the liver was significantly greater than in either of the other two groups.

Table XXXVI

Fibrinogen level and plasma fibrinolytic activity in patients with hepatocarcinoma and in each of the control groups
The results are expressed as Means ± S.E.M.

Subjects	No.	Fibrinogen (mg. per 100ml.)	Plasma fibrinolytic activity (Percentage of fibrin lysed per hour)
Cirrhosis of liver	60	253 ± 9.6	9.4 ± 1.1
		(P = <0.05>0.02)	(P = <0.001)
Healthy	60	284 ± 9.6	0.5 ± 0.04
		(P = <0.001)	(No significant difference)
Hepatocarcinoma	35	450 ± 19.3	0.5 ± 0.06

The fibrinogen level in the patients with hepatocarcinoma was higher than that in the healthy controls which in turn was higher than that in the controls with cirrhosis of the liver.

Response to Adrenaline

The response to the subcutaneous injection of adrenaline (0.5 ml. of of 1/1000 solution) in 20 patients with hepatocarcinoma in 8 healthy controls and in 17 controls with cirrhosis of the liver are set out in Table XXXVII. No significant change in fibrinolytic activity followed the injection of adrenaline in the patients with hepatocarcinoma whereas significant increases were encountered in both control groups. The increase in the controls with cirrhosis of the liver was significantly greater than in the healthy controls.

Table XXXVII

Plasma fibrinolytic activity induced by adrenaline in patients with hepatocarcinoma and in each of the control groups
The results are expressed as Means ± S.E.M.

Subjects	No.	Plasma fibrinolytic activity (Percentage of fibrin lysed per hour)		
		Spontaneous	20 minutes after adrenaline	Increment
Healthy	8	0.3 ± 0.08	3.8 ± 0.8 (P = <0.001)	3.6 ± 0.8 (P = <0.001)
Cirrhosis of liver	17	4.1 ± 0.9	34.8 ± 5.0 (P = <0.001)	30.8 ± 3.9
Hepatocarcinoma	20	0.4 ± 0.8	0.6 ± 0.9 (No significant difference)	-

Response to Ischaemia

The response to ischaemia (Experiment 1, Chapter 3) in 20 patients with hepatocarcinoma in 20 healthy controls and in 35 control patients with

Table XXXVIII

Plasma fibrinolytic activity induced by ischaemia in patients with hepatocarcinoma and in each of the control groups
The results are expressed as Means ± S.E.M.

Subjects	No.	Plasma fibrinolytic activity (Percentage of fibrin lysed per hour)		
		Spontaneous	Following ischaemia	Increment
Healthy	20	0.5 ± 0.05	15.8 ± 2.5 (P = <0.001)	15.3 ± 2.8 (No significant difference)
Cirrhosis of liver	35	6.4 ± 0.8	24.6 ± 2.0 (P = <0.001)	18.2 ± 2.0
Hepatocarcinoma	20	0.4 ± 0.06	0.6 ± 0.1 (No significant difference)	-

Table XXXIX

Spontaneous and induced plasma fibrinolytic activity before and after the development of hepatocarcinoma in 4 patients with cirrhosis of the liver

Case No.	Before development of carcinoma				After development of carcinoma			
	Fibrinogen (mg./100 ml.)	Plasma fibrinolytic activity (Percentage of fibrin lysed per hour)			Fibrinogen (mg./100 ml.)	Plasma fibrinolytic activity (Percentage of fibrin lysed per hour)		
		Spontaneous	Following adrenaline	Following ischaemia		Spontaneous	Following adrenaline	Following ischaemia
1	307	5.1	33.3	28.1	395	0.5	0.5	0.6
2	267	3.2	33.3	30.3	353	0.4	0.5	0.3
3	192	10.1	66.6	16.6	485	0.4	0.4	0.7
4	181	18.6	66.6	33.3	428	0.3	0.8	0.6

cirrhosis of the liver are set out in Table XXXVIII. No significant change in fibrinolytic activity was encountered in the patient with hepatocarcinoma whereas an increase occurred in each of the control groups. No difference emerges on comparison of the increments encountered in each of the control groups.

Before and after Development of Hepatocarcinoma

Four patients have been investigated before and after development of the hepatocarcinoma (Table XXXIX). Prior to the development of the tumour increased spontaneous fibrinolytic activity was encountered in all four and there was significant response to adrenaline and following ischaemia. After development of the carcinoma spontaneous fibrinolytic activity was within normal limits and the response to adrenaline and following ischaemia were abolished.

Response to Paravenous Stimuli

The responses to the paravenous injection of adrenaline (10 µg.) and of 5-HT (1 µg.) each in 5 patients with hepatocarcinoma (Chapters 4 and 5) are set out in Table XL. Also set out in this table are the findings in

Table XL

Plasma fibrinolytic activity following the paravenous injection of adrenaline and 5-HT in patients with hepatocarcinoma and in healthy controls
The results are expressed as Mean ± S.E.M.

Stimulus	Patients with hepatocarcinoma		Healthy Controls	
	No.	Plasma fibrinolytic activity (Percentage of fibrin lysed per hour)	No.	Plasma fibrinolytic activity (Percentage of fibrin lysed per hour)
Adrenaline (10 µg.)	5	3.3 ± 0.7	20	11.0 ± 0.9
5-HT (1 µg.)	5	6.7 ± 0.5	5	26.5 ± 3.9

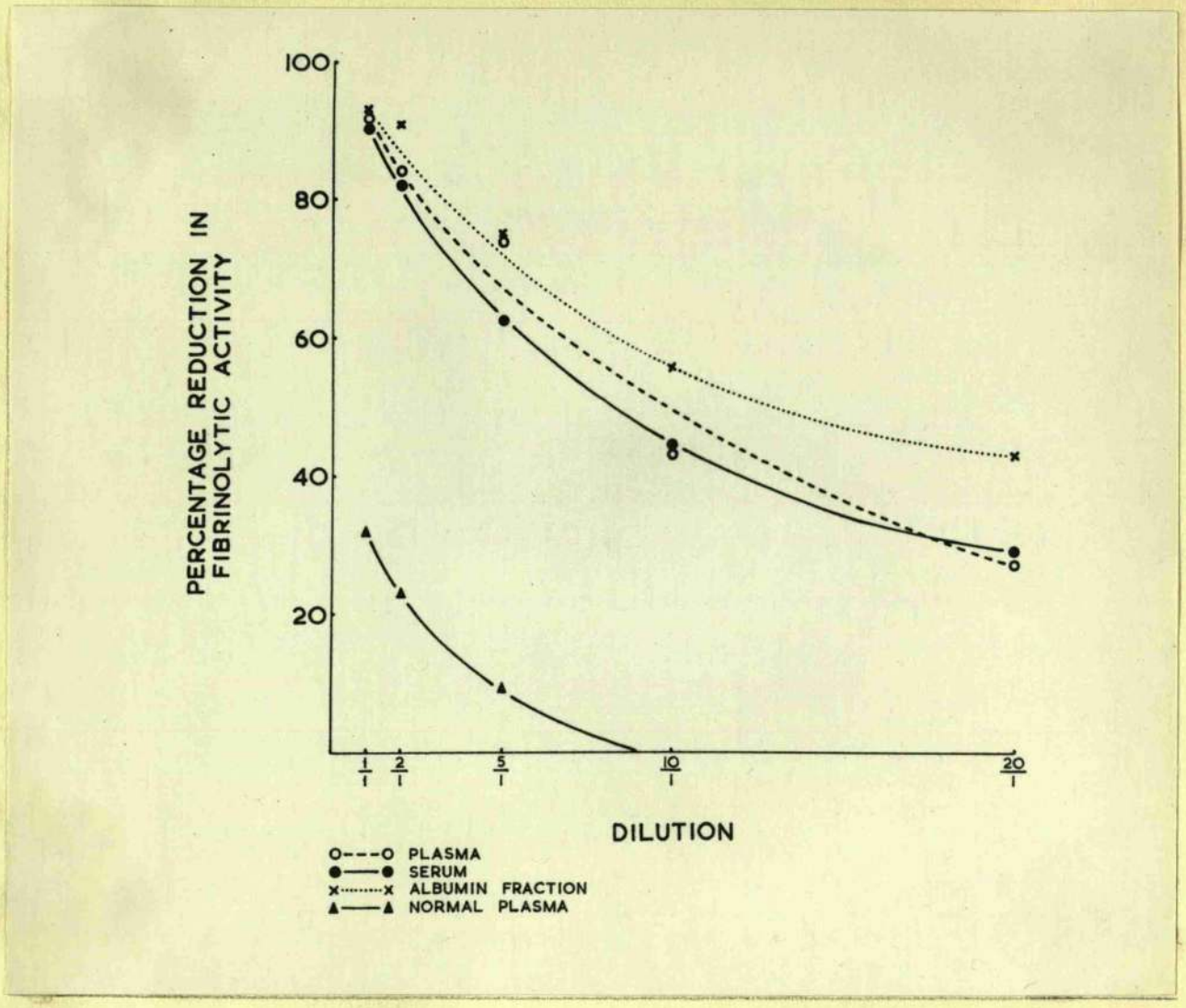

Figure 9. Mean 'antifibrinolytic' activity of the plasma, of serum and of the albumin fraction of plasma from 12 patients with hepatocarcinoma together with the mean 'antifibrinolytic' activity of the plasma from 5 healthy controls.

healthy controls. Fibrinolytic activity was induced in the 5 patients but the intensity was significantly lower than in healthy controls ($P = < 0.001$ in each case).

'Antifibrinolytic' Activity

The results of the investigation of the 'antifibrinolytic' activity of plasma, of serum and of the albumin fraction of the plasma from 10 patients with hepatocarcinoma together with the activity of plasma from 5 healthy controls are set out in Table XLI and depicted graphically in the Figure 9. While normal plasma was possibly possessed of some inhibitory effect this was highly significantly less than that exerted by the plasma

Table XLI

'Antifibrinolytic' activity of plasma, serum and albumin fraction of plasma in 10 patients with hepatocarcinoma. The 'antifibrinolytic' activity of the plasma in 5 healthy controls is included

Fraction	Mean percentage reduction in fibrinolytic activity ± S.E.				
	Ratio of fibrinolytic plasma/Test fraction				
	1/1	2/1	5/1	10/1	20/1
Plasma	90 ± 3	83 ± 5	63 ± 9	44 ± 10	30 ± 8
Serum	91 ± 4	83 ± 5	74 ± 6	43 ± 9	27 ± 7
Albumin	92 ± 4	91 ± 5	74 ± 9	55 ± 12	43 ± 11
Normal Plasma	32 ± 4	24 ± 4	9 ± 5	-	-

from the patients with hepatocarcinoma. All 3 fractions of the patients' blood were possessed of 'antifibrinolytic' activity even in the highest dilution (1/20). There is no significant difference on comparison of the activity of individual fractions at each dilution employed. With each fraction the inhibition exerted was consistent with itsbeing a function of the concentration of the inhibitory substance.

Antiplasmin Activity

The antiplasmin activity of 3 specimens of plasma each showing marked 'antifibrinolytic' activity and of 3 specimens from healthy controls were determined. There was no significant difference between the two groups.

'Antifibrinolytic' Activity of the Tumour

The results are set out in Table XLII. In each case the saline extract of the tumour exerted significantly greater effect than that of normal liver.

Table XLII

'Antifibrinolytic' activity of a buffered saline extract of primary hepatocarcinoma and of a normal liver

Source of inhibitor		Percentage of fibrin lysed per hour					
		Ratio of fibrinolytic plasma to inhibitor					
		1/0	1/1	2/1	5/1	10/1	20/1
Hepatocarcinoma	1	11.1	0	0	0	5.0	5.0
	2	16.7	0	0	0	5.0	5.0
Normal liver	1	25.0	16.7	16.7	25.0	25.0	25.0
	2	11.1	8.3	8.3	10.0	11.1	11.0

Other Malignant Tumours

The response to adrenaline and following ischaemia were determined in 19 patients with various malignant tumours details of which are set out in Table XLIII. Both responses were abolished in 8 of the 19 patients. Six of the 19 had metastases in the liver and in 3 of these both responses were abolished.

Table XLIII

Plasma fibrinolytic activity in response to adrenaline and to ischaemia in other malignant tumours

Tumour	Metastases to liver		Response to adrenaline and ischaemia	
	With	Without	Inhibited	not inhibited
Bronchogenic carcinoma	1	5	1	1 4
Carcinoma of stomach	4	2	3	1 2
Carcinoma of colon	1	1	1	1
Sarcoma		2	1	1
Lymphosarcoma		3	2	1

Comment

The experiments on rabbits establish that the inhibition of the response of veins to 5-HT, the removal of 5-HT from platelets and the inhibition by atropine of the mechanism which imparts fibrinolytic activity to plasma results in the formation of red blood clot within veins in circumstances which, in the normal untreated rabbit, would result in a local platelet thrombus only. The subsequent lysis within 24 hours of 3 of the 8 thrombi in Experiment 34 suggests, for the reasons advanced in Chapter 10, that histamine, the action of which is not inhibited by corticotrophin, may well be responsible.

The observations on patients show that there develops in the albumin faction of the plasma of patients with hepatocarcinoma a factor, the addition of which to plasma showing spontaneous fibrinolytic activity, that is with increased content of activator, results in inhibition of that

activity. This is in contrast to normal plasma which under these circumstances, as first reported by Bidwell (1), possesses little inhibitory effect. Since the plasma of patients with hepatocarcinoma does not differ from normal plasma in inhibiting plasmin it may be concluded that the factor probably inhibits the activator present in the fibrinolytic plasma thereby preventing the conversion of plasminogen to plasmin during clotting. The appearance of this inhibitor with the development of hepatocarcinoma may be considered responsible for the disappearance of the spontaneous fibrinolytic activity encountered in uncomplicated cirrhosis of the liver. It has been shown that the paravenous injection of adrenaline and of 5-HT will still induce fibrinolytic activity of the plasma within the segment of vein employed although the intensity of this activity is significantly less than in healthy controls. In these circumstances the amount of activator produced must be greater than the limited quantity of inhibitor present in the plasma within the segment of vein. It would seem reasonable to suggest that the apparent failure of patients with hepatocarcinoma to respond to ischaemia and to the intramuscular injection of adrenaline is not a failure of either of these stimuli to produce response but is due to the inhibitor nullifying the expression of that response, namely the ultimate development of fibrinolytic activity. In other words proactivator is probably converted to activator which however is inhibited.

Although an inhibitor of activator similar to that occurring in hepatocarcinoma cannot be demonstrated in significant quantity in normal plasma it has been encountered in high concentration in the plasma of blood obtained at laparotomy from the splenic vein of apparently normal spleens (7). This is not the only source of such activity for Kwaan, Lo and McFadzean (6), extending observations on patients with increased spontaneous plasma fibrinolytic activity previously reported (9) have found that in the response to adrenaline the fibrinolytic activity of specimens of blood obtained from the hepatic veins was significantly lower than that of specimens obtained either from the inferior vena cava below the inflow of the hepatic veins or

from the superior vena cava. This they found to hold in the splenectomised subject. It would appear therefore that the liver exerts antifibrinolytic activity. Although its mode of so doing is unknown it may well be by the production of antiactivator. It should be noted that Macfarlane and Biggs (11) in an investigation of the 'antifibrinolytic' activity of extracts of various organsfound the highest activity in those of liver and spleen. There is experimental evidence that the liver exerts antifibrinolytic activity, for example, Nolf (15) described the occurrence of intense fibrinolytic activity in the hepatectomised dog following the injection of peptone. However if the liver was only temporarily excluded from the general circulation the peptone-induced fibrinolysis was inhibited once the circulation through the liver was restored.

The source of the antiactivator in hepatocarcinoma has not been determined. It must either develop in response to the carcinoma or be derived from it. If it is the former then the most likely origin is the residual liver tissue. The course of the disease results in a progressive reduction in liver tissue yet the only change encountered has been an increase in the anti-activator activity. It is possible therefore that the source is the carcinoma itself. The finding that a saline extract of the tumour is possessed of antiactivator activity significantly greater than normal liver tissue is consistent with the source being the tumour.

While in a cirrhotic patient other complications may result in the temporary disappearance of spontaneous fibrinolytic activity from the plasma (9) none of these inhibit the response to adrenaline or to ischaemia. In such patients the absence of increased fibrinolytic activity of the plasma coupled with a failure to respond to adrenaline and to ischaemia thus far has proved diagnostic of hepatocarcinoma and has been encountered even in the early phase of development of the tumour. In so far as other forms of malignant tumours are concerned our observations are incomplete and conclusions may not be drawn. The results however indicate that other forms of malignancy both with and without metastases to the liver may be

associated with the occurrence of 'antifibrinolytic' activity in the plasma, presumably due to the presence of antiactivator.

It would seem reasonable to conclude that the presence of antiactivator in the plasma of patients with hepatocarcinoma with consequent prevention of development of fibrinolytic activity may be responsible for the thrombosis of subcutaneous veins encountered in 11% of such patients (13).

The increased level of fibrinogen in hepatocarcinoma is of interest. It has been suggested that the integrity of the vascular wall normally may be dependent upon a process of fibrin formation and that the rapid turnover of prothrombin, platelets and fibrinogen support this suggestion. By feeding S^{25}-labelled dl-methionine or yeast, Madden and Gould (10) found the 50 percent turnover rate of fibrinogen to be 5-6 days. Gitlin and Borges (5) reported a similar result from observations on 2 patients with congenital afibrinogenaemia. It is tempting to suggest that the increased levels of fibrinogen encountered in patients with hepatocarcinoma is due to a reduced turnover, a consequence of inhibition of the fibrinolytic mechanism. In this connection it is of interest that McFadzean and Yeung (14) have found that the prothrombin levels in hepatocarcinoma are within normal limits and are significantly higher than in uncomplicated cirrhosis. Further in 5 patients who developed hepatocarcinoma in a cirrhotic liver the prothrombin levels previously significantly below normal became normal.

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CONCLUSIONS

The purpose of this research primarily was to find answer to the two questions posed in the Introduction: "Can fibrinolytic activity be stimulated in the plasma of blood within veins?" and "Could fibrin have been formed and lysed in the venules obstructed by platelet thrombi?" These may be answered in the affirmative.

It has been shown that fibrinolytic activity can be induced within subcutaneous veins in man by the stimulation of what would appear to be a cholinergic effector mechanism present in their walls. Such stimulation reflexly produces fibrinolytic activity in other veins of the same limb and also in veins of the contralateral limb through the agency of similar effectors. The 'receptors' of this reflex seem to be widely distributed in tissue for they can be stimulated by intradermal injection. The effectors have not been identified. It seems reasonable to suggest that, on stimulation, they secrete lysokinase which converts proactivator in the plasma to activator which, if fibrin is formed, converts plasminogen to plasmin with consequent lysis of fibrin. The foregoing mechanism has been shown also to exist in the veins of the ears of rabbits.

Evidence consistent with a similar mechanism occurring in arteries has been presented.

Set out in Table B are the stimuli thus far known to induce fibrinolytic activity within veins. Acetylcholine, the effector substance,

Table B

Stimuli known to induce fibrinolytic activity in man and in the rabbit

Group 1		Group 2
Cold (15°C)	Arrest of arterial supply	Histamine
Adrenaline		Priscol
Noradrenaline		Heat (40°C)
Pituitrin		Inflammation
5-HT		
Procaine		

is not included. Save that 5-HT acts upon receptors readily paralysed by LSD 25, their modes of stimulation of the cholinergic effector have not been established. It has been suggested that stimuli of Group 1, by virtue of their action as vasoconstrictors, produce relative ischaemia and when applied over, alongside or within a vein they act by constricting the vasa vasorum thereby producing relative ischaemia of the vein wall. It has been argued that histamine may be the common denominator to stimuli of Group 2 and that histamine, under the conditions of the experiments, may produce relative ischaemia within the 'wheal' response. It follows, mayhap, that ischaemia is the common denominator to all stimuli known to excite, indirectly, the cholinergic effector mechanism.

Evidence has been presented consistent with the 5-HT, liberated from a platelet thrombus, augmented by the action of histamine, similarly liberated, being capable of inducing within veins fibrinolytic activity of such intensity as to lyse fibrin as rapidly as it can be formed. It is concluded that a platelet thrombus, with its attendant vasoconstriction, is of itself sufficient to secure haemostasis in certain injuries to arteries and veins and that the more cumbersome and disrupting mechanism of intravascular clotting is to be avoided or restricted. This is achieved by the action of 5-HT augmented by that of histamine, released from the agglutinated platelets, stimulating the cholinergic effector mechanism in the vessel wall with consequent development of fibrinolytic activity within the vessel and lysis of any fibrin formed.

It has been shown in the rabbit that, if the 5-HT is removed from platelets or if the response by veins to 5-HT is inhibited, red blood clot forms within veins in response to an injury which, in the untreated animal, would result in the formation of a local platelet thrombus only. It has also been shown in man that there is a high incidence of thrombosis of subcutaneous veins in hepatocarcinoma. In such patients it has been demonstrated that antiactivator activity develops in the plasma. It is concluded that the raison d'être of the fibrinolytic mechanism is to

maintain veins and arteries free from fibrin.

It has been shown in the rabbit that both corticotrophin and trauma inhibit the lysis of thrombi produced experimentally within veins and that this is due to the inhibition of the response of veins to 5-HT. The responses to histamine and adrenaline are not inhibited. These observations may have an application in the study of thrombo-embolic phenomena in man, notably those following operation.

The feeding of cholesterol to the rabbit results in the inhibition of the lysis of thrombi experimentally produced within veins. It has been shown that the response to 5-HT and to adrenaline are inhibited but the precise nature of the inhibition has not been determined. These observations may have an application in the study of the genesis of atheroma in man. There are two major and apparently conflicting hypotheses concerning the aetiology of atheroma. Among many others, Keys (6) emphasises the importance of dietary fats whereas Duguid (2), whose observations have been confirmed (1, 3, 5) holds that the initial phase of development of atheroma is the occurrence of mural thrombi. The inhibitory influence of lipaemia on fibrinolytic activity may well reconcile the two apparently conflicting hypotheses (4).

It has been found that exercise of muscles in a limb rendered ischaemic prevents the development of increased fibrinolytic activity in blood subsequently flowing through the limbs. Evidence has been presented consistent with this form of inhibition being due to an inhibitory effect exerted upon the cholinergic effectors in the walls of arteries.

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